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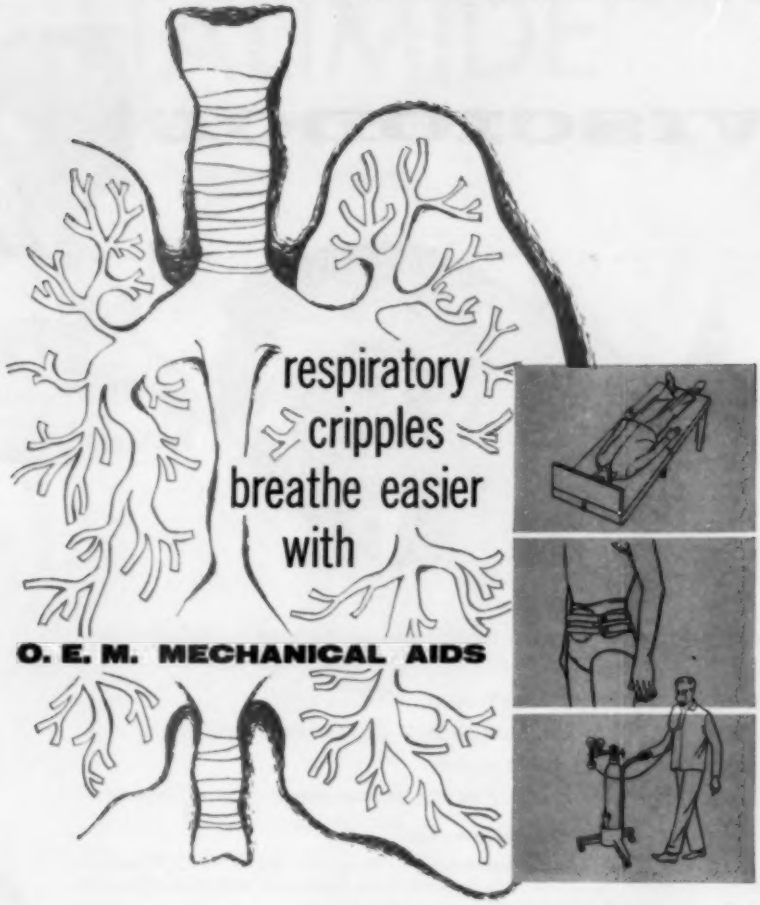

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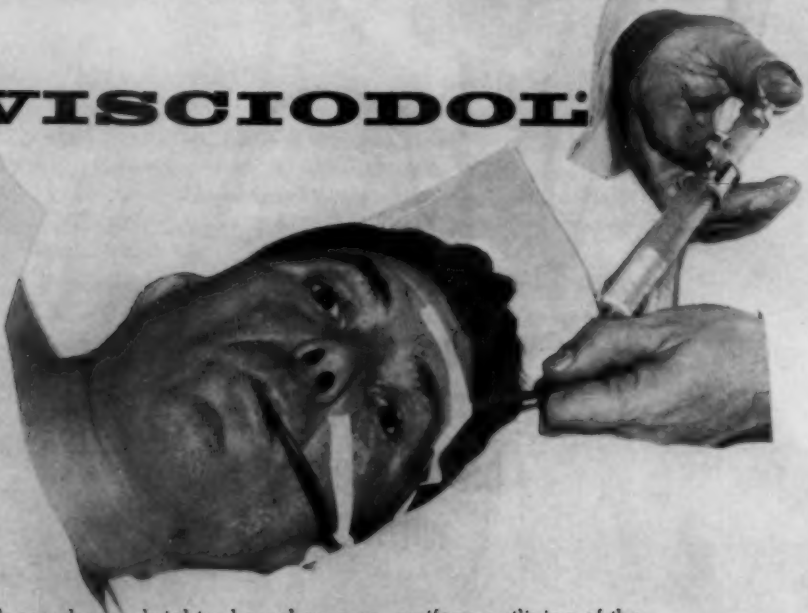

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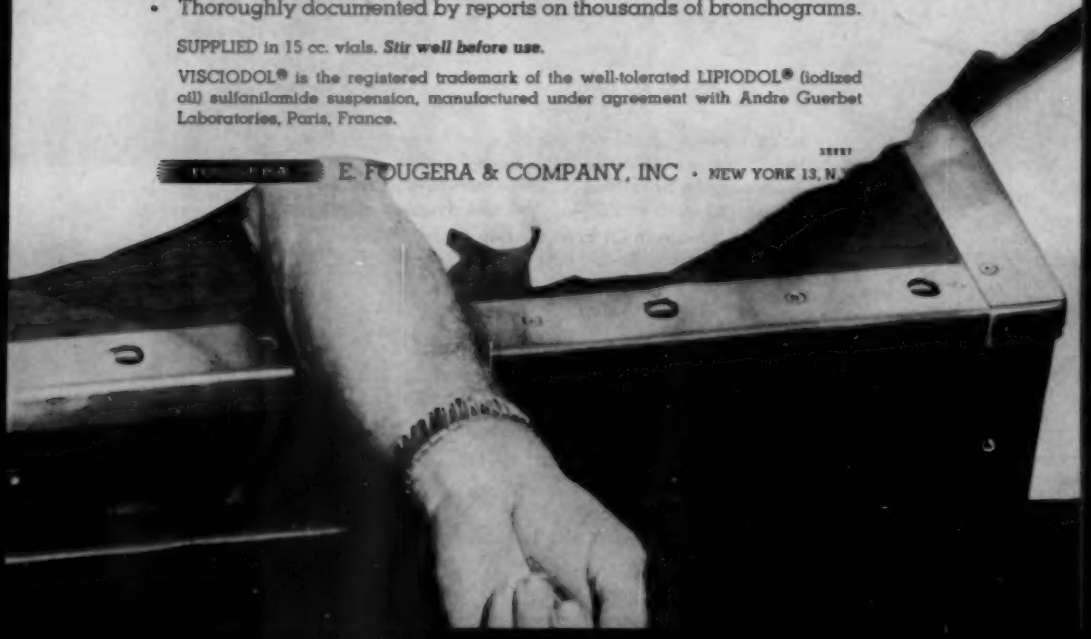

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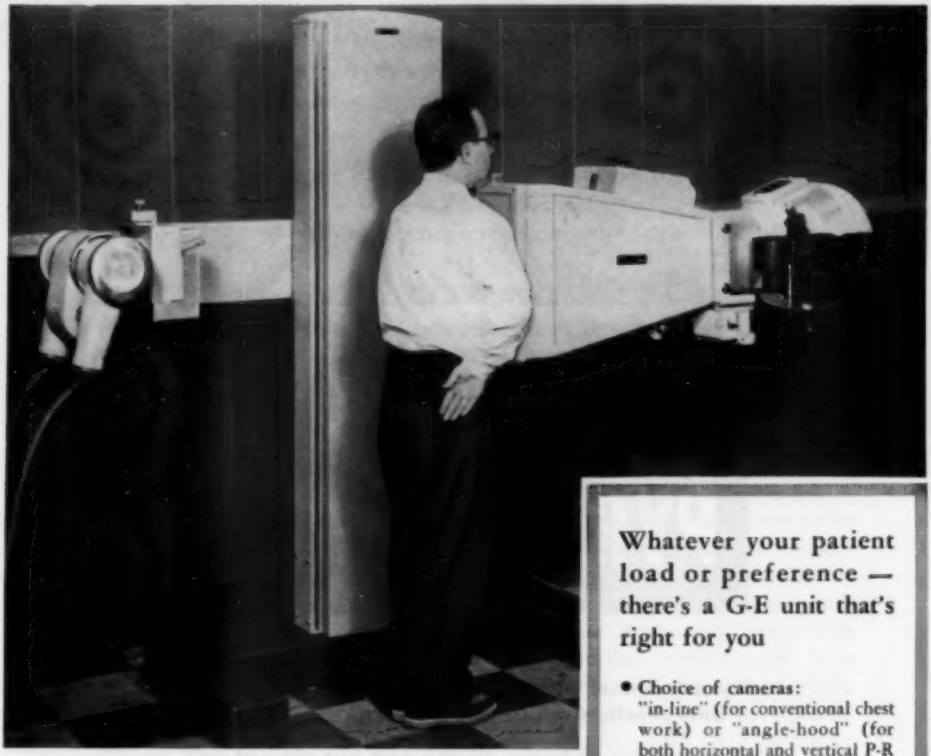

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 76 *November, 1957* NUMBER 5

BCG: PAST, PRESENT, AND FUTURE[1]

ARVID J. WALLGREN[2]

(Received for publication May 9, 1957)

I presume that Calmette and Guerin's statement that they had succeeded in suppressing the virulence of an originally virulent bovine strain of tubercle bacilli was viewed sceptically by numerous bacteriologists throughout the world. Was it possible to produce such a profoundly persistent change of bacillary qualities and characteristics only by changing the culture's nutritional and environmental conditions? We learn from genetics that acquired characteristics are not transmitted by heredity. How then did BCG emerge from a strain of virulent bovine tubercle bacilli? Hitherto it has not been possible to restore its original virulence by cultivating BCG on substrates more suitable for virulent bovine tubercle bacilli or by repeated passage through susceptible animals.

The avirulence of BCG has apparently become fixed by heredity. This problem has become much more acute following the commonplace experience with resistant strains of tubercle bacilli which emerge during chemotherapy. It is probable that BCG developed in similar fashion by a radical mutation which occurred by cultivating the strain on media unsuitable for ordinary tubercle bacilli but suitable for mutants, and that the virulent bacilli were gradually more and more effectively suppressed until the mutant strain outgrew them. Those outside of France who studied BCG thirty years ago or read about such studies will perhaps remember that a few investigators, among them Petroff, claimed that they were able to dissociate virulent tubercle bacilli from BCG. Some years later this was not possible. Apparently the early cultures of BCG were not completely deprived of virulent strains, which subsequently disappeared.

BCG is certainly not a uniform, unchangeable avirulent strain of tubercle bacilli. Its potency may vary and the reaction produced by a BCG vaccine prepared in one laboratory is often not identical with that of a vaccine from another

[1] The first R. J. Hatfield Lecture, sponsored by the Potts Memorial Institute, Livingston, New York, presented at the annual meeting of the National Tuberculosis Association, Kansas City, Missouri, May 9, 1957. Dr. Esmond R. Long's introduction of Dr. Wallgren is presented in the American Trudeau Society section of this number of the REVIEW, pp. 920, 921.

[2] Emeritus Professor of Pediatrics, Royal Caroline Medical School, Stockholm, Sweden; Chief, Pediatric Clinic, Karolinska Sjukhuset, Stockholm, Sweden.

laboratory. One cannot talk about *the* BCG, but about various *strains* of BCG. This must be kept in mind when evaluating BCG vaccination.

Calmette and his co-workers held the view that BCG was nonpathogenic for even the most susceptible animals. We know now that this is not true. BCG may give rise to disease in laboratory animals, e.g., hamsters and mice, whose local or general resistance has been deliberately decreased by different methods. It is proper, therefore, to ask if the present BCG under all circumstances will remain nonpathogenic for man. I will return to this question later.

The first reports on the efficiency of BCG vaccination were based on studies that could not stand critical analysis, and some investigators ascribed an exaggerated evaluation to BCG not only as an immunizing agent against tuberculosis, but also against several other diseases. Needless to say, such unscientific optimistic reports have done much to impede a true evaluation of BCG. These reports were especially uncritical, and the categoric statements regarding BCG "premunization" were not founded on sound scientific research and were contradicted by common clinical experience.

It was claimed that BCG, given by mouth to newborn infants, produced immunity without producing tuberculin sensitivity and that immunity developed almost immediately. Reports of the fate of BCG "premunized" and other children were published in order to establish the prophylactic value of BCG, but the registered diagnoses of fatal cases in vaccinated as well as in nonvaccinated series were not accepted in scientific circles. It was quite natural that these excessive statements met with sound scepticism and occasioned bitter criticism. There is no wonder that the whole idea of BCG vaccination at that time was regarded in some quarters more or less as a fraud and that the medical world in general did not accept BCG as a valuable weapon in antituberculosis campaigns. Frankly speaking, the original BCG work was not taken seriously.

BCG vaccination was rejected in most countries and, eventually, only in France and in countries influenced by French medical practice was the peroral "*Calmettization*" of newborns practiced.

Some years elapsed before BCG vaccination was revived and put on a more scientific basis. The principle then followed was to simulate as far as possible a primary tuberculous infection, i.e., BCG vaccination should be performed in such a manner as to produce tuberculin sensitivity after a reasonable incubation period. Because the increased resistance, if this developed at all after BCG vaccination, probably was not acquired before tuberculin sensitivity was demonstrable, it was considered important to prevent the occurrence of tuberculous infection before allergy was established and to remove and segregate children living in a tuberculous environment until vaccination had taken effect. It was also regarded as unnecessary and even harmful to inject BCG into an already tuberculin-positive subject, i.e., an infected person who had already acquired by virulent infection the specific immunity that the BCG vaccination was supposed to afford. Thus, prevaccination tuberculin testing was regarded as necessary, as well as the segregation of contact cases until they were proved tuberculin negative after a reasonable incubation period after the last exposure (and

until the tuberculin test had become positive after the incubation period following BCG vaccination).

In the beginning, only infants threatened by tuberculous infection were vaccinated in this way. Later on vaccination was extended to older children and, finally, to children and adolescents without evident risk of exposure.

Because BCG given orally—the method of Calmette—failed to produce tuberculin sensitivity or, if it sometimes did, this occurred very late, another method of vaccination was preferred. In order to prevent too strong a local reaction, a superficial intradermal injection of a convenient dose was chosen and gradually became the method of choice for BCG vaccination. The peroral BCG vaccination, however, is still used in a few countries. In Brazil, de Assis of Rio de Janeiro and his associates introduced a modified form of oral vaccination, the so-called "concurrent vaccination." They give giant doses of BCG repeatedly every month for six months without any preliminary tuberculin testing, even to infants who are exposed to the disease. The Brazilians claim that this method of vaccination is very effective and free from complications. So far this method has not been studied systematically elsewhere, and the results have not been verified in other countries.

BCG vaccination became very popular in Scandinavia where it had had its revival. Gradually, in Sweden a more general mass vaccination has been performed in newborns and in schoolchildren. In both of these age groups it has been convenient to vaccinate because almost all infants are born in maternity hospitals and there is compulsory tuberculin testing of schoolchildren, and the vaccinations are performed by the medical personnel at the respective institutions.

In some countries lyophilized BCG is used instead of the fresh vaccine. There are doubtless some advantages to be gained through facilitating storage and distribution of the vaccine and also making its potency more uniform. Researches now in progress have shown thus far that freeze-dried BCG may be used effectively at least one year after production, but with a certain decrease of its potency. It would be premature to recommend its use, at least in countries with no transportation difficulties.

At present there are some "BCG-promoting" countries and some "BCG-nonpromoting" countries in the world and it would be unfair to place the United States elsewhere than in the second group. Except in a few places, i.e., Chicago, where the production is under Rosenthal's direction, and Albany, where the production was formerly under Birkhaug's direction, most cities and states are not in favor of BCG vaccination; in a few places like Minneapolis a campaign has been conducted against BCG vaccination. Only a few serious attempts have really been made in the United States either to prove or disprove the efficacy of BCG prophylaxis. Among these I refer to the study performed by Aronson and his associates among the Indians on reservations and in Alaska.

One can fairly well understand reluctance to promote BCG vaccination in the States as well as elsewhere, and several reasons for such an attitude are suggested: (*1*) The uncritical appraisal of the effectiveness of the vaccination and

the exaggerated evaluation mentioned earlier have not been forgotten; (*2*) the pretended or real local and general complications; and (*3*) the rapid decrease of the tuberculosis morbidity and mortality rates in many states without any BCG vaccination at all.

With regard to the degree of efficacy of BCG vaccination, I will quote only one new experience. A study which I regard as a true model is that conducted by the Tuberculosis Study Committee of the British Medical Research Council. This study was planned most carefully in order to obtain conclusive results and has so far evaded all of the unscientific loopholes which mar almost all similar studies.

In advance of the study, without knowing anything about the prospects, the parents gave permission to have their children vaccinated with BCG. All children were fourteen and a half to fifteen years of age. Those who turned out to be tuberculin positive were excluded from the study, and 14,000 of the 27,000 tuberculin-negative subjects chosen at random were vaccinated intradermally with BCG, and the other 13,000 were not vaccinated and were used as a control group. All of the children were examined very carefully both clinically and roentgenographically at repeated intervals, and erroneous evaluation of the tuberculous processes was avoided in every way. The first report published more than a year ago (two and a half years after vaccination) showed a definite difference in the tuberculosis morbidity of the vaccinated and the unvaccinated groups (vaccinated group, 0.37 per year per 1,000; unvaccinated group, 1.94 per year per 1,000), the morbidity being five times less in the former group. Of special interest and importance was the development of 6 cases of miliary tuberculosis and tuberculous meningitis in the unvaccinated group compared to no case among the vaccinated children. The Committee draws the conclusion that BCG vaccination has increased the resistance against tuberculosis in a substantial way.

I might also refer to the big experiment going on in Puerto Rico conducted by Carroll Palmer and his associates which includes children from one to eighteen years of age. After four years the vaccinated group had only two-thirds as much tuberculosis as the tuberculin-negative controls. I suppose that even Palmer, who certainly cannot be regarded as an enthusiastic advocate of BCG vaccination, will now admit that his own study on a susceptible population has shown the practical value of the vaccination and that it may be of benefit.

No one who has had personal experience with adequately performed BCG vaccination can challenge seriously the increased resistance to tuberculosis which it can produce. I suppose that it might be said that there is rather unanimous agreement that an adequately performed BCG vaccination, that is, a vaccination that has had time to produce tuberculin sensitivity before exposure, undoubtedly provides fairly satisfactory protection: (*a*) against the immediate consequences of a tuberculous infection and against serious forms of primary tuberculosis; (*b*) against early postprimary diseases, miliary tuberculosis, and tuberculous meningitis, as well as against pleurisy and early postprimary pulmo-

nary tuberculosis. The protection against late postprimary types of tuberculosis seems to be slighter and more uncertain.

We have as yet no simple test which determines when immunity has been established and its degree. The tuberculin reaction which is usually employed as a test of specific immunity is in fact a test of the degree of sensitivity. Because the immunity and hypersensitivity to tuberculin are supposed to develop almost concurrently after a virulent infection, the tuberculin reaction has been used as a test for the existence of immunity. It is well known, however, that the degree of tuberculin sensitivity cannot be used as a measure of the degree of immunity. A rather high degree of tuberculin sensitivity may be present without any demonstrable immunity and vice versa. In practice, it is possible to use the tuberculin test to determine the existence of immunity; a positive test after virulent infection or BCG vaccination indeed indicates the existence of immunity, and the immunity is supposed to last as long as tuberculin sensitivity is demonstrable. Lacking other easily applied methods of assessment, we have to rely upon tuberculin sensitivity when we wish to determine the existence of immunity. Another factor that may interfere is the occurrence of subclinical tuberculous infections, which may influence the persistence of the immunity. With these reservations, it might be said that the immunity is established six to eight weeks after parenteral vaccination and that it can disappear within one year; but, on the other hand, according to our experience, it generally lasts for as long as seven to ten years, perhaps more. The duration of the immunity thus varies considerably and it is not possible to tell in advance how long it will last. As a rule, it will last sufficiently long to be of real benefit to a child exposed to tuberculous infection.

In experiments with radioactive BCG, my associate Lars Ström has shown that tuberculin-positive subjects behave quite differently from tuberculin-negative persons; radioactive BCG remains much longer at the site of injection in tuberculin-positive reactors, moves more slowly toward the regional lymph nodes, and is destroyed much more rapidly—to judge from the quick urinary excretion of the isotope—than is the case in the tuberculin-negative uninfected and unvaccinated persons. That this difference is not due to tuberculin hypersensitivity is shown by the fact that people who have been vaccinated repeatedly with BCG and remain tuberculin negative, because they seem to lack the faculty of tuberculin reaction, nevertheless behave as do those who have become tuberculin positive after vaccination. These persons apparently possess immunity without having developed tuberculin sensitivity.

The likelihood of BCG vaccine's producing local complications after inoculation is quite variable. Batches of vaccine from one laboratory may behave quite differently in this respect from those of another laboratory, although both vaccines may seem adequately efficient from an immunizing point of view. Importance is therefore attached to the strain of BCG used as vaccine and also to the manner in which the vaccination is performed, inasmuch as more abscesses are produced by subcutaneous injection than by the intracutaneous or percutaneous routes. The Swedish vaccine (Selander) produces almost no local com-

plications even in the newborn although the same dose is used as in adults, while the Danish vaccine in the newborn produces local lymph node abscesses in 20 per cent when the adult dose is used. This figure is reduced to 0.25 per cent with one-fourth of the ordinary dose of the vaccine (Biering-Sörensen, Brit. M. J., 1955, No. 4947, 1048).

These local abscesses may be very inconvenient, but they are never dangerous because they remain localized and always heal spontaneously although often after a long delay. They heal more rapidly after curettage. By and large, these local complications have played only a minor role in the campaign against BCG vaccination, although one can readily understand the unwillingness of a mother to have a second child vaccinated if a previous one has been troubled with abscesses in the regional lymph nodes.

Of somewhat more importance but considerably less frequency is the risk of developing lupus at the site of the BCG scar. Even this disease has healed with adequate treatment and without untoward danger for those vaccinated.

Complications after BCG vaccination should be carefully studied, but such investigations have not always been made. It is especially important that this be done when tuberculous disease occurs after vaccination. BCG does not afford an absolute protection against tuberculosis any more than does any other kind of vaccination against some specific disease. It is not at all unusual for people to fall ill with tuberculous disease after infection, although they have been satisfactorily vaccinated. Besides, numerous cases of tuberculous disease in BCG-vaccinated persons are recorded in whom the vaccine had not had the opportunity to exert any protective effect because the subject was already infected when vaccinated or the infection had occurred during the incubation period after BCG vaccination. These types of tuberculous disease (after tuberculous infection) are, of course, not to be considered to be due to the vaccination or as failures of protection.

Illnesses other than tuberculous diseases after BCG vaccination have often been ascribed to BCG without any reasonable cause. There is almost no type of disease which has not been imputed to the injection of BCG. Because there is still much heated discussion about BCG vaccination, the diseases erroneously believed to result from BCG vaccination have played and are still playing an important role in the anti-BCG propaganda. A survey of these reports has not given any support to the apparent relationship.

Bacteriologic examination of tissues from such diseases apparently resulting from BCG vaccination have usually disclosed that the bacilli that produced the disease were virulent strains distinct from BCG—except in a few cases. Time does not permit a detailed description of these cases and I will give only a few comments on them.

In all, 5 such cases were reported, 4 in children and one in an adolescent. All of these persons were vaccinated in an adequate manner, and many vaccinations were performed at the same time with the same batch of vaccine without any untoward reactions. It is therefore impossible to say that the diseases were due to inadequate vaccination or to too virulent vaccine; they must have been due

to an increased susceptibility to BCG of the vaccinated person. In these cases the disease produced by BCG developed through a hematogenous spread of the BCG while all other types of complication from BCG are produced by pathologic reactions at the site of injection and in the regional lymph nodes by lymphogenous spread of the BCG. The lesions produced have been localized in the lungs, the skeleton, and in the distant lymph nodes. In 4 of the cases the lesions led to the death of the vaccinated persons. Careful bacteriologic studies of the type of bacilli isolated from the patients proved that they corresponded exactly to the characteristics of BCG.

Recently H. Gormsen has reported histologic examination of persons who had been vaccinated with BCG. In 13 of 20 children who had died in accidents from six weeks to forty months after vaccination there were signs of lesions in internal organs which were apparently due to hematogenous spread of the BCG. In these cases the lesions had not produced any manifest disease.

The knowledge that serious systemic diseases due to BCG may arise in exceptional cases, even if the vaccine and the vaccination procedure are adequate, must be taken into consideration in the discussion of the advantages and disadvantages of BCG vaccination.

The degree of risk of clinical disease from such systemic complications cannot be foreseen and it is not possible to foretell if a child about to be vaccinated is one of those abnormally susceptible to BCG. Even if only 5 such cases are known to have occurred among the many millions of BCG vaccinations, it is a catastrophe for the individual who becomes the victim of such a complication. It is surprising that these reports have come only from the Scandinavian countries with their very low tuberculosis morbidity and generally good natural resistance. Certainly, it cannot be due solely to the great frequency of vaccination. If the same proportion of systemic complications had occurred in other countries, we should have had many more of such cases reported. May it depend upon the more careful and skilful bacteriologic study of these inflammatory lesions performed in the Northern European countries?

An important argument against a more general BCG vaccination program is the declining rate of tuberculosis morbidity and the extent to which this has been attained. It may roughly be determined by tuberculin testing of schoolchildren in countries in which until now BCG vaccination has not been more generally performed. The lower the tuberculosis morbidity, the fewer the ambulatory sources of infection and the smaller the number of children infected. From the 100 per cent of positive reactors at fifteen years of age at the turn of this century in Europe, at the present time one observes all transitions down to a few per cent of positive reactors in many countries with a well-organized effective antituberculosis health service and a satisfactorily resistant population. In such areas—for example, in the present state of Minnesota—it would be out of the question to introduce mass BCG vaccination. Certainly there is no need for such a measure; and excessive public health measures should not be applied.

A tendency prevails everywhere in the civilized world today to underrate a country's risk of tuberculosis, while there is an exaggerated opinion about the

risk of some other diseases. Look at poliomyelitis and the propaganda for vaccination against it and the very slight interest in the United States in BCG vaccination. Yet, using Dubos's figures, we can reckon with morbidity and mortality rates about ten times higher in tuberculosis than in poliomyelitis.

Tuberculosis still exists as a disease even in Minnesota, and in selected cases an artificially induced resistance against tuberculous infection would seem highly desirable. This applies particularly to tuberculin-negative pupil nurses or medical students, to persons who work in hospitals for tuberculous patients, or to small children who are uninfected contact cases in tuberculous families.

If the general prophylactic public health measures against tuberculosis are adequate, the morbidity rate low, and the sources of infection are so rare that only a few children become infected at school age, no further prophylactic measures seem necessary; in such a community, general BCG vaccination is not indicated. In a community with a very high tuberculosis morbidity rate, the sources of infection are numerous, the risk of exposure is very great, and the majority of children become infected, many of them at an early age.

Because the prognosis of tuberculous infection is more serious when the younger child becomes infected, it is necessary to introduce all available prophylactic measures as early as possible. We have examples of such communities in East Asia. For several years WHO and UNICEF have conducted a gigantic vaccination program in these countries. Childhood tuberculosis, however, is still very common, and during my visit to the pediatric departments of ten medical centers in India in January and February of this year I had ample opportunity to observe several hundreds of small children suffering from tuberculous meningitis. WHO's principle has been to vaccinate only children more than one to two years of age, not infants. More and more children in India are born in maternity hospitals or in MCH centers, in some places up to 90 per cent, and it should be recommended that all of these newborn infants be vaccinated. In a country such as India everything should be done to fight tuberculosis on all fronts, and BCG vaccination is a valuable adjuvant which should be used as early as possible. It is a false notion, however, that BCG vaccination alone would solve the problem of tuberculosis in India—an opinion that I heard expressed many times, probably due to an exaggerated propaganda.

When the question arises of employing mass BCG vaccination in a country with a very high tuberculosis morbidity rate, the risk of exposure and the danger of primary infection are so great that an eventual risk of complications of the vaccination does not play any considerable role and need not be stressed. It is quite different when vaccination is used in a country with a very low morbidity rate; in such a country—for instance, in Sweden—mass vaccination is performed on people who will be exposed only in exceptional instances; and the increased resistance produced by BCG will only in very unusual cases be of benefit to the vaccinated individual. Thus, most vaccinations are unnecessary. If there were some danger inherent in the procedure itself, this danger would be increased many times in the eyes of the vaccinated person or of his relatives. The risk of serious complications of BCG vaccination should, of course, always be bal-

anced against the degree of risk which a susceptible individual runs of acquiring severe tuberculous disease after infection.

The disadvantage of BCG vaccination with regard to the elimination of tuberculin tests as a very important method for the diagnosis of tuberculous disease should not be forgotten in this connection. Before starting mass BCG vaccination, this disadvantage should also be balanced against the degree of the general exposure risk to susceptible persons in the country.

When BCG vaccination was first introduced into a country where there was no previous experience of its efficiency, it was at first tentatively given to selected groups of the population considered to be at high risk of developing tuberculosis. After its benefit was demonstrated, it was extended to larger and larger groups. This seems to be a logical development of BCG vaccination in a country with a high tuberculosis morbidity rate. The reverse development, i.e., diminishing the use of general vaccination and continuing vaccination only of selected groups when the tuberculosis morbidity has decreased to a very low rate, should be logical too. When only a small percentage of children become infected at fifteen years of age, as in Sweden now, there seems to be little indication to continue with general vaccination, for instance, of the newborn.

Even in countries with very low morbidity rates there will probably always be persons who run a great and very evident risk of exposure, e.g., medical students, nurses, hospital personnel, and contact cases; in these subjects there will be an indication for increasing the resistance by means of BCG vaccination or any other as efficient or more efficient method of producing active immunity that may be available in the future.

There is another factor, however, which may influence the future use of BCG as a prophylactic measure. That is the availability of other more efficient and simpler methods of prophylaxis. At present it may not be feasible to make any definite conjecture because we lack sufficient knowledge of experimentation in human beings; however, promising results have already been obtained in experimental animals. I refer to the use of isoniazid in tuberculous infection for the purpose of preventing tubercle bacilli from producing pathologic lesions. The recent studies made by Carroll Palmer and his associates are well known to all of you. These studies have shown that it is possible to destroy a large proportion of a population of tubercle bacilli by repeated doses of isoniazid during the incubation period of the tuberculous infection. Isoniazid can apparently convert a massive inoculation of virulent tubercle bacilli into a controlled benign tuberculous infection. The animals become sensitive to tuberculin and they acquire some resistance against subsequent new infections.

It is not possible at present to tell whether this type of chemoprophylaxis will also work in humans. French writers Lelong and co-workers claim that they have been able to prevent tuberculous infection in infants. They could not prove, however, that a tuberculous infection really had occurred. What they have shown is that isoniazid-treated and heavily exposed infants remained tuberculin negative and healthy.

Dr. Canetti of the Pasteur Institute has advocated the administration of

isoniazid-resistant BCG concurrently with isoniazid to all exposed and still uninfected children. They would thus be protected from tuberculous disease manifesting itself before the vaccination immunity has had time to develop and would build up a specific immunity, unhampered by the simultaneous administration of isoniazid. So far we have had no experience of this procedure and we do not know if it will be possible to utilize it in practice.

Several clinicians have tried to prevent tuberculosis by intense isoniazid treatment of early primary tuberculosis. The opinions regarding the effect of isoniazid on primary tuberculosis are divided. Some clinicians are very optimistic, while others have seen no more rapid decrease of the lesions than occurs without any specific treatment. The opinion is more unanimous on the possibility of preventing postprimary hematogenous spread of the disease by treatment of the primary infection with isoniazid. As you know, there is a well-planned collective study going on in the United States by the Public Health Service with the collaboration of many pediatric clinics and childrens' hospitals. When this study is terminated, we will certainly know where we stand regarding the use of isoniazid as a prophylactic measure in primary infection.

The French National Institute of Hygiene is busy with a similar study. The French writers claim that they have already observed definite benefits of this treatment. It is well known that, of course, it is not possible to prevent the development of hematogenous tuberculous disease by chemotherapy in all cases of primary tuberculosis. Only time and the most carefully controlled studies will show what can be done by means of this prophylactic method.

Isoniazid in primary tuberculosis does not give rise to resistant strains, with some few exceptions. In a collective statistical study of more than 700 children with primary tuberculosis or meningitis treated with isoniazid, it was possible to isolate a resistant strain of low virulence only twice.

* * *

I am now at the end of my lecture, and will add only a few words of conclusion about the future of BCG vaccination. Any attempt on my part to prognosticate the future of BCG would be mere guesswork because several factors are bound to influence and determine the concept, value, and practical use of BCG in years to come: the degree of natural resistance in the future population, the efficiency of new available methods of prophylaxis, the exposure risk, and the tuberculosis morbidity rate. There will probably be a gradual introduction of BCG vaccination in selected groups in many places where there has been reluctance to start vaccination. There will probably also be a further development of the vaccination in countries with high morbidity rates where they have not yet availed themselves of the benefit of the vaccination to its full extent, for instance, in India. The more tuberculosis morbidity decreases in a country with a previously high prevalence of tuberculosis, the less will be the need for mass vaccination, while vaccination of selected severely exposed subjects will continue. If in the distant future, tuberculosis—thanks to a very high natural resistance —develops into a benign infection and seldom produces clinical illnesses even

in the most susceptible persons, then BCG vaccination of selected groups will be unnecessary and BCG vaccination will play no further part in the anti-tuberculosis campaign. However, if wars and/or other disasters occur and produce a decrease in the standard of living and in the general resistance of the population, this may give rise to a new increase of tuberculosis morbidity and also to a change in the prevailing attitude toward BCG vaccination, with a revival of its application.

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REPORT OF AD HOC ADVISORY COMMITTEE ON BCG TO THE SURGEON GENERAL OF THE UNITED STATES PUBLIC HEALTH SERVICE

A committee invited by Surgeon General Leroy E. Burney, to examine the Public Health Service policy and program in the field of BCG vaccination, met in Washington, June 14, 1957. Dr. Otis L. Anderson, Chief of the Bureau of State Services, acting as the Surgeon General's representative, asked the Committee to formulate a new statement and make new recommendations, if it seemed appropriate, on the use of BCG vaccination as a public health procedure and on further research as to its value. At Dr. Anderson's request the Committee selected one of its own group as chairman of the meeting.

A previous advisory committee considered the question of BCG vaccination in September, 1946, and a statement on the use of BCG, consistent with the recommendations of that committee, was issued by the Public Health Service in August, 1950. In this statement the Public Health Service took the position that additional research on the effectiveness of the vaccine was required, that indiscriminate use of BCG might divert attention from other control measures, that mass BCG vaccination programs were not indicated in this country, and, finally, that vaccination should be limited to persons with unusually great exposure to tuberculous infection.

It appeared to the present Committee that a logical evaluation of BCG vaccination and the formulation of recommendations on the policy of the U. S. Public Health Service could be made only after consideration of (*1*) the present status of the tuberculosis problem as a whole, (*2*) facts on BCG vaccination that have become available since the last statement issued by the Public Health Service, (*3*) the contribution that might be expected from BCG in diminishing the problem in the light of the new facts, and (*4*) the advantages and disadvantages of various public health applications of BCG.

The Committee recognized that nearly all aspects of the tuberculosis problem have undergone great changes during the past decade. The mortality rate has decreased from about 30 per 100,000 in 1946, when the previous advisory committee met, to less than 10 in 1956. The morbidity rate has also declined, although apparently not so rapidly. At the same time the consequences of tuberculosis morbidity have been lessened by the effectiveness of the antimicrobial agents, particularly isoniazid. The Committee members agreed that further research on the effectiveness of antimicrobial drugs in first infection is desirable. It was noted that the infection rate, as reflected in numerous tuberculin testing surveys, has reached a new low. While precise information is not available, it is probably safe to say that in the United States as a whole *less* than one-half of 1 per cent of the population are being newly infected with tubercle bacilli each year, although the rates are higher than this in certain areas. However, the available evidence indicates that in all areas the risk of infection is decreasing at an accelerating rate.

The basic premise for use of BCG vaccine is that the changes produced in the host by vaccination will protect in some measure against the hazards resulting from tuberculous infection. It is generally accepted that vaccination confers no benefit on persons who have already been infected, and that its use should, therefore, be restricted to noninfected persons, i.e., nonreactors to tuberculin. If, as widely believed, the protection afforded by vaccination tends to wane with the passage of time, the ideal period for vaccination is a few months prior to exposure to tuberculosis. Ordinarily it is not possible to predict exposure so accurately; in certain occupational groups, on the other hand, probable exposure can be assumed.

When vaccination is offered to general population groups, it has been customary to limit it to the younger segments, in which there has been as yet little opportunity for infection. In practice, vaccination is carried out after tuberculin testing. Among the very young, in the United States, the frequency of reaction to tuberculin is low, and lowest in these in an environment in which exposure is minimal. Since in the early years of life substantial changes in environment are relatively infrequent, freedom from exposure in the immediate future can be predicted for the majority of those found negative. In older age groups subjected to tuberculin testing, the proportion of reactors to tuberculin is usually higher, so that fewer persons in the group are eligible for vaccination. Recent well-controlled studies have shown that, with the passage of time, tuberculous disease is more likely to develop among those found positive at the time of tuberculin surveys than among those not reacting. In other words, the greatest hazard in a surveyed group at any age exists among those found positive to tuberculin and who, therefore, would not be vaccinated. It was pointed out that, accordingly, chief attention from the point of view of tuberculosis control should be devoted to those found to react to tuberculin in any survey.

It was recognized as obvious, however, that any measures that could be taken to prevent development of infection in the first place should be part of every tuberculosis program.

The value of BCG vaccination in controlling tuberculosis in the United States has been a matter of controversy in recent years. The Committee recognizes arguments both for and against its more general application. Several studies have brought forth convincing evidence that vaccination in man with a strain of BCG known to be of high potency leads to some degree of increased resistance against tuberculous disease. The duration of such resistance has not been defined, but several reports indicate a lower incidence of development of tuberculosis in vaccinated than in control groups several years after vaccination. It has been postulated that in such groups the effect of vaccination is reinforced by subsequent small spontaneous infections which the vaccinated persons overcome. The Committee noted, however, that accurate studies have not been made on the efficacy of revaccination with BCG, and believed that, on the whole, present information is inadequate on the persistence of increased resistance from BCG vaccination. The members believed that BCG appears to be as safe as other vaccines

in common use, but noted that several cases of progressive disease and death attributable to BCG have been reported.

In considering disadvantages attendant upon use of BCG vaccination, the Committee members stressed the value of the tuberculin reaction as an index of exposure to tuberculosis. Since BCG vaccination, in order to be considered satisfactory, converts nonreactors into reactors to tuberculin, the procedure makes it impossible to use the tuberculin test (*1*) as evidence of recent infection in the individual, (*2*) as an index of infection in population groups, (*3*) for the location of sources of contagion, (*4*) as a preliminary screening device prior to chest roentgenographic examination in the diagnosis of tuberculosis, or (*5*) for differential diagnosis in diseases with some similarity to tuberculosis.

These considerations were believed to be of increasing importance in the light of the current continuing decline in the prevalence of infection and manifest increasing concern over the hazards of excessive radiation by X-rays. The Committee agreed that, if the protection afforded by vaccination were absolute and persistent, the loss of the tuberculin test for all these purposes would be outweighed by the protective effect of the vaccine, but since all studies indicate that the protection is imperfect, the loss of the index value of the tuberculin test appeared significant.

The Committee expressed the opinion that vaccination may lead to a false sense of security which could result in failure to observe precautions that otherwise would be taken.

The Committee gave careful attention to the numerous investigations carried out since the 1946 conference on BCG vaccination, which have shown a wider range of variation in the reported efficacy of BCG than was evident at that time. A range of efficacy from 0 to 80 per cent among study populations eligible for vaccination has been reported from various controlled studies. It was noted that in almost all of the several reports on the follow-up of large groups of persons vaccinated with BCG some cases of disease and death from tuberculosis are recorded among vaccinated persons. In one of the studies in which the highest efficacy was recorded, the investigation reported by the Tuberculosis Vaccines Clinical Trials Committee to the Medical Research Council in Great Britain, in which vaccination was carried out with the Danish official BCG vaccine, there were 13 cases of definite tuberculosis in a vaccinated population group of 14,100 within two and a half years after initiation of the follow-up. In the U. S. Public Health Service investigation in Puerto Rico, carried out with the BCG vaccine produced by the New York State Department of Health, 78 cases of tuberculosis occurred among approximately 50,000 vaccinated persons in five and a half years. These figures indicate that the degree of protection afforded by the vaccine is far from absolute. In the Medical Research Council study, the morbidity rate among the vaccinated was only one-fifth of that reported for a nonvaccinated group of 13,200 studied concurrently; in the Public Health Service study, the rate was two-thirds of that recorded for some 27,000 controls con-

currently studied. A striking finding in both studies was a high subsequent incidence of tuberculosis morbidity in persons strongly sensitive to tuberculin at the time of survey who were not eligible for vaccination and therefore not in either the vaccinated or the control group.

Of equal pertinence to the task of the Committee were the results of the Public Health Service's trial of BCG vaccination in Muscogee County, Georgia, and Russell County, Alabama, in which vaccination was carried out with the BCG vaccine produced by the Research Foundation in Chicago, Illinois. In this community study, there was no statistically significant difference in the tuberculosis developing among vaccinated and nonvaccinated persons after six years of observation. Furthermore, the tuberculosis that developed among persons who were eligible for vaccination by the ordinary criteria was an extremely small fraction of the total tuberculosis problem of the community during the follow-up period.

The members of the Committee were particularly impressed by the apparent variability in vaccinating potency of different BCG strains, as shown by laboratory studies as well as by the diversity of results in large field trials. It was noted that critical analytical studies have been made of the value of only a small number of the "lots" of BCG vaccine that have been used, and that no agreement has been evident as yet in different research groups on the choice of BCG strains for vaccination. It was believed that the significance of apparent variations in potency of strains, with respect to the induction both of tuberculin allergy and resistance to infection, is not yet understood, and that the implications of such variations have not yet been taken sufficiently into account in current studies. As one member of the Committee expressed it, "the label BCG does not define a product of predictable and standard properties and activity."

In considering the public health aspects of BCG vaccination programs the Committee noted that the tuberculosis mortality rates for the white population in the United States, in which vaccination has been practiced on a very small scale only, are much like those in white populations abroad, in which vaccination has been carried out on an extensive scale. They were even more impressed by the fact that reductions in mortality have been as great in certain European countries that do not practice BCG vaccination as in countries of comparable size and economic state in which BCG vaccination is widely used.

With all of these considerations in mind the Committee did not believe that a categorical statement on the degree of protection afforded by BCG vaccination could be made.

The Committee, while unanimously agreeing that BCG confers some protection against tuberculosis, considered at length whether or not the amount of protection provided by vaccination is sufficient to justify greater use of BCG in the United States in the light of current general progress in tuberculosis control and in the face of the several disadvantages noted. In the absence of consistent and convincing evidence of persisting protection and in the light of a

greatly decreased risk of infection and its consequences, the Committee expressed the opinion that the use of BCG should be determined by the particular circumstances obtaining in a local community or population group. This determination should be based on a wide variety of factors, among the most important of which will be the strength of the tuberculosis program as a whole, the prevalence of tuberculosis in the community at the time, and the probable risk of infection in the future. In general, the question will come up for decision chiefly in groups or communities where exposure is high and weakness in other means of control is recognized. It was believed by the Committee that the responsible officials in such communities and groups should be sufficiently well informed on the advantages and disadvantages of BCG vaccination, through currently available reports, to make an intelligent decision.

The Committee is convinced that large-scale BCG vaccination programs, including routine vaccination of the newborn, are not indicated in this country. It is believed, however, that the advantages of vaccination outweigh the disadvantages for tuberculin-negative persons who are exposed to a definite risk of infection, especially if they cannot be retested frequently with tuberculin. Under certain local circumstances, the following individuals and groups are examples of suitable subjects for BCG vaccination:

1. Physicians, nurses, medical and nursing students, laboratory workers and hospital employees. (It was noted, however, that if a hospital has established an adequate tuberculosis control program very little exposure to tuberculosis will occur in that institution.)
2. Persons unavoidably exposed to continued contact with infectious cases of tuberculosis in the home.
3. Patients, inmates and employees of institutions such as mental hospitals and prisons, in which case-finding programs indicate that exposure to tuberculosis is likely to be high.

The Committee noted the fact that in September, 1956, after review of material similar to that studied by the present Committee, the Committee on Administrative Problems of the American Trudeau Society decided that the current statement of the Society, formulated in April, 1949, was not in need of revision. That statement recommended use of BCG vaccine in groups similar to those listed in the foregoing paragraph, and recommended continued emphasis on general measures of tuberculosis control. The Committee noted other recent statements of similar import by a number of investigators and groups with long experience in BCG vaccination.

The Committee finally considered the desirability of further studies in the field by the Public Health Service. It is the consensus of the Committee that, in view of the apparent low risk of tuberculosis among present nonreactors in the United States, new investigations on the value of BCG cannot be made without excessively large study populations. For this reason, the Committee believes it is unwise to recommend that the Public Health Service initiate new vaccination trials. However, it recommends strongly that the controlled studies on population

groups already started be carried on for as long a period as they furnish significant information on the rates of development of tuberculosis in the vaccinated and control groups now under observation.

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THE EFFECTS OF EARLY ISONIAZID TREATMENT IN EXPERIMENTAL GUINEA PIG TUBERCULOSIS[1]

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INTRODUCTION

The recent investigations of Ferebee and Palmer (1) and Palmer, Ferebee, and Hopwood (2) on the use of isoniazid to prevent the development of serious tuberculous disease in guinea pigs have aroused considerable discussion and controversy (3). Other investigators have already reported studies related to the preventive use of isoniazid (4–6), and the Public Health Service Study of Palmer and associates is continuing (2).

The present studies were planned to expand the observations of the Public Health Service group by introducing a few additional variables and utilizing some different techniques in determining the effectiveness of the very early administration of isoniazid on experimental tuberculosis in guinea pigs. The design of these experiments differs from that of the Public Health Service group in the following ways: Two infecting dosages of tubercle bacilli, one large and one small, were employed for challenge, rather than just one large infecting dose, and these were injected subcutaneously rather than intraperitoneally; a fixed large dosage of isoniazid, injected intraperitoneally, was used rather than variable dosages of the drug in drinking water; the effectiveness of treatment was measured not only by survival, post-mortem pathologic changes, and the tuberculin reactions, but also by culture of the organs of the surviving and sacrificed animals (isoniazid-susceptibility and catalase-activity tests were performed on the cultures positive for *M. tuberculosis*); the differences in susceptibility to reinfection were determined for both a tuberculin-positive and a tuberculin-negative group of guinea pigs which survived massive infection as a result of isoniazid therapy.

MATERIALS AND METHODS

Grouping of Animals

Nine groups of random-bred guinea pigs, each containing 60 animals, were studied. These guinea pigs, either white or light colored, were received in the laboratory in two shipments about a week apart. The animals were observed in the laboratory for at least one month after arrival to attempt to exclude any spontaneous infection and to bring them up to a desired weight before including them in the experiments. The animals were kept in metal cages in groups of 10 and were fed oats, carrots, and lettuce. Only guinea pigs weighing 300 to 350 gm. were included. The guinea pigs were tuberculin tested three to seven days before starting the experiments; all were tuberculin negative.

The nine groups of guinea pigs were divided as follows:

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[1] From the bureaus of Laboratories and Tuberculosis, New York City Department of Health, 125 Worth Street, New York, New York.

Control groups:

Control No. 1: Challenged with a relatively small number of tubercle bacilli (2×10^3 bacterial units), but not treated with isoniazid

Control No. 2: Challenged with a large number of tubercle bacilli (3×10^6 bacterial units), but not treated with isoniazid

Control No. 3: Neither challenged with tubercle bacilli nor treated with isoniazid

None of the animals in any of the three control groups received intraperitoneal injections of saline as a control measure for the intraperitoneal injections of isoniazid given to the treated animals.

Experimental groups:

Group A: Treated with isoniazid for five days prior to challenge with a relatively *small* number of tubercle bacilli; isoniazid treatment continued for *six weeks* after challenge

Group B: Treated with isoniazid for five days prior to challenge with a *large* number of tubercle bacilli; isoniazid treatment continued for *six weeks* after challenge

Group C: Treated with isoniazid for five days prior to challenge with a *large* number of tubercle bacilli; isoniazid treatment continued for *twelve weeks* after challenge

Group D: Treated with isoniazid for five days prior to challenge with a *large* number of tubercle bacilli; isoniazid treatment continued for *fifty weeks* after challenge

Group E: Received no isoniazid prior to challenge with a *large* number of tubercle bacilli; isoniazid treatment *started simultaneously* with challenge and continued for *twelve weeks*

Group F: Received no isoniazid prior to challenge with a *large* number of tubercle bacilli; isoniazid treatment *started fourteen days after* challenge and continued for *twelve weeks*

Challenging Technique

Three different guinea pig-virulent, isoniazid-susceptible, and catalase-active cultures of tubercle bacilli were used which had been isolated from 3 previously untreated patients with far advanced active pulmonary tuberculosis. The results with these 3 patient-strains were so similar that for the purpose of the present report they have been considered as a single strain.

Guinea pigs were infected by subcutaneous injection into the inguinal region. Each animal in control *Group No. 1* and experimental *Group A* (challenged with a relatively *small* number of organisms) received 0.3 ml. of a diluted culture suspension of *M. tuberculosis* containing 6×10^3 bacterial units per ml. or a total of approximately 2×10^3 *bacterial units*. Each animal in control *Group No. 2* and in experimental *Groups B, C, D, E,* and *F* (challenged with a *large* number of organisms) received 0.3 ml. of a diluted culture suspension of *M. tuberculosis* containing 9×10^6 bacterial units per ml., or a total of approximately 3×10^6 *bacterial units*.

The two amounts of bacterial units were determined by dilution of washed bacterial suspensions in phosphate buffer (pH 7.0) and by adjusting each photometrically in a Coleman Jr. spectrophotometer to an optical density corresponding to about 6×10^3 and 9×10^6 bacterial units per ml., respectively, as previously determined by plate counts.

Drug Administration

Isoniazid was administered by intraperitoneal injection, each dose consisting of the equivalent of 5 mg. of drug per 100 gm. of body weight dissolved in distilled water (1 mg. of isoniazid in each 0.1 ml. of distilled water). The amount of drug injected was varied to correspond with the weight of each animal during the experiments. The animals were

weighed every four weeks. Animals treated prior to challenge received isoniazid daily for five days before infection with tubercle bacilli. After infection, all of the treated animals received isoniazid injections three times a week (Monday, Wednesday, and Friday).

Tuberculin Testing

All infected guinea pigs were tuberculin tested at five- to six-week intervals until death or sacrifice. An attempt was made to avoid using the same site for repeated tuberculin tests. The tests, when given, were located in different, previously unused sites on the flanks and abdomen. Tuberculin tests were given intradermally with 0.1 ml. of a 1:100 dilution of Old Tuberculin[2] and were read forty-eight hours later. The widest portion of the area of induration and erythema was measured in reading the first test (five to six weeks after infection). The average initial reaction measured 14 mm. in diameter. For the purposes of the present study, it was decided that reactions measuring 6 to 20 mm. or more were to be considered strongly positive; reactions measuring from 2 to 5 mm. in diameter were recorded as weakly positive (the average weakly positive reaction was 4 mm.); and reactions less than 2 mm. in size (usually no reaction or a small prick mark) were interpreted as negative.

Drug treatment was stopped on guinea pigs which became tuberculin negative after having been tuberculin positive. Such negative reactors were separated from their original groups, placed in individual cages, and tuberculin tested at the usual intervals until death or sacrifice.

Culturing of Viscera

At the time of death or sacrifice of each animal in both the control and treated groups, a careful autopsy was performed for any gross evidence of tuberculosis, and cultures of the spleen, liver, and lungs were obtained irrespective of the gross findings.

The culture technique consisted of inoculating two tubes of egg medium with each organ emulsion made in phosphate buffer (pH 7). The emulsions were also examined by direct microscopy for acid-fast bacilli. Cultures were recorded as positive or negative for tubercle bacilli, irrespective of the amount of growth. Isoniazid-susceptibility and catalase-activity tests were done on one positive culture from each animal, usually the one isolated from splenic tissue, using the methods described in a previous report (7) except for one modification, i.e., the isoniazid-susceptibility tests were done on the primary cultures (indirect method) rather than on the organ emulsions (direct method).

Plan of Studies

Study 1—Survival time: Survival curves were established for each of the three control groups of animals and for the six infected and treated groups extending over an observation period of thirty-six weeks after challenge.

Study 2—Tuberculin testing: This was carried out in all surviving animals in the nine groups for a period of thirty-six weeks following challenge. Results were tabulated at two observation points, at twenty-four weeks and thirty-six weeks, respectively, after challenge.

Study 3—Culturing of viscera: Viscera of each animal of all nine groups were examined and cultured at the time of natural death, or at sacrifice thirty-six weeks after challenge, with the following exceptions:

(*a*) From control *Group No. 3*, 13 animals, healthy and tuberculin negative after thirty-six weeks of observation, were removed for *Study 4*.

(*b*) From *Group C*, 6 animals, tuberculin negative at twenty-four and thirty-six weeks after infection, were removed for *Study 4*.

(*c*) From *Group D*, 7 animals, tuberculin negative at twenty-four and thirty-six weeks after infection, were removed for *Study 4*, while the remaining survivors were sacrificed at *fifty weeks* after infection.

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[2] Manufactured by Eli Lilly Co., Indianapolis, Indiana.

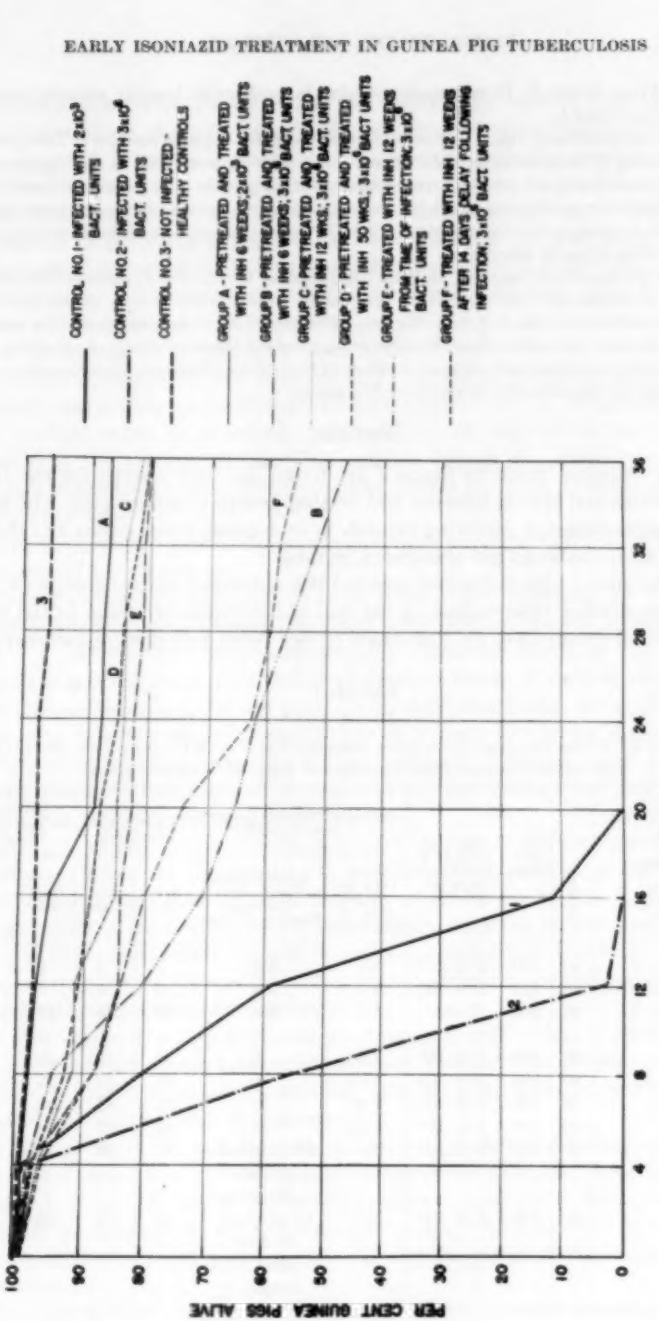

Fig. 1. Chart showing survival for nine groups of 60 animals each

(*d*) From *Group E*, 13 tuberculin positive but otherwise healthy animals were removed for *Study 4*.

Study 4—Reinfection of tuberculin-positive and tuberculin-negative survivors: Two groups, each containing 13 animals previously infected and treated, were used in this experiment. One group, consisting of strongly tuberculin-positive guinea pigs, was obtained from *Group E*. The other group, composed of tuberculin-negative guinea pigs, came from *Groups C* and *D*. All animals were challenged with 3×10^6 bacterial units of *M. tuberculosis* forty-one weeks after primary infection.

Thirteen previously uninfected animals from control *Group No. 3*, observed to remain tuberculin negative over the same forty-one weeks, were simultaneously challenged with the same inoculum to serve as a control group. The effects of reinfection on each of the two groups of animals were determined by observing survival times to death of all of the animals and by the anatomic and cultural findings in their visceral organs, in comparison with these findings in the primarily infected control group.

Results

Study 1—survival time: In figure 1 are found survival curves for the three control groups and the six infected and treated groups of guinea pigs. The numbers and percentages of surviving animals in each group twenty-four and thirty-six weeks after challenge are also shown in table 1.

All of the guinea pigs in the two infected but untreated control groups (*Nos. 1* and *2*) were dead of tuberculosis by the end of the twentieth week. In the uninfected control group (*No. 3*), 3 animals (5 per cent) had died of intercurrent,

TABLE 1

STUDY 1

SURVIVAL OF 3 UNTREATED AND 6 ISONIAZID-TREATED GROUPS OF GUINEA PIGS AT 24 WEEKS AND AT 36 WEEKS AFTER CHALLENGE WITH M. TUBERCULOSIS

Animal Group	Number	Per Cent	Bacterial Units of *M. Tuberculosis* Injected	Isoniazid (5 mg. per 100 gm.) Intraperitoneally		Survivors 24 Weeks After Challenge		Survivors 36 Weeks After Challenge	
				Given Daily for 5 Days Prior to Challenge	Given 3 Times Weekly After Challenge *Duration in Weeks*	Number	Per Cent	Number	Per Cent
Control No. 1	60	100	2×10^3	No	No	0	*0*	0	*0*
Control No. 2	60	100	3×10^4	No	No	0	*0*	0	*0*
Control No. 3	60	100	None	No	No	*59*	*98*	*57*	*95*
Group A	60	100	2×10^3	Yes	6	53	*88*	53	*88*
Group B	60	100	3×10^4	Yes	6	37	*62*	28	*47*
Group C	60	100	3×10^4	Yes	12	51	*85*	49	*82*
Group D	60	100	3×10^5	Yes	50	52	*87*	48	*80*
Group E	60	100	3×10^4	No	12 (started at time of challenge)	50	*83*	48	*80*
Group F	60	100	3×10^4	No	12 (started 14 days after challenge)	37	*62*	33	*55*

nontuberculous disease by the end of the thirty-sixth week of observation. The largest percentage of surviving animals (88 per cent) was found in *Group A*, the one composed of guinea pigs which were infected with the smaller number of organisms and treated, after pretreatment, for only six weeks. Almost as many survivors (80 to 82 per cent) were found in *Groups C*, *D*, and *E*. The smallest percentage of surviving animals occurred in *Group B* (47 per cent), while the percentage of surviving animals in *Group F* (55 per cent) was only slightly larger.

For those animals massively infected and treated without delay for at least twelve weeks, pretreatment (*Groups C* and *D*) did not seem to offer any additional advantage over treatment started simultaneously with infection (*Group E*). Delay of treatment for fourteen days following infection, however, contributed appreciably to an increase in mortality (*Group F*). Treatment for less than twelve weeks in massively infected animals resulted in an impressive decrease in the number of surviving animals (*Group B*), while increasing the duration of treatment beyond twelve weeks (*Group D*) failed to alter significantly the survival time.

In general, weight gain in the animals which survived for thirty-six weeks was quite similar for all groups.

Study 2—tuberculin testing: Five to six weeks after infection, all of the surviving animals in control *Group No. 2* and in the isoniazid-treated groups infected with 3×10^6 bacterial units were strongly tuberculin positive. The surviving animals in control *Group No. 1* and 58 of the 60 in *Group A*, both of which groups were infected with 2×10^5 bacterial units, were also tuberculin positive at six weeks; the two remaining animals in *Group A* were tuberculin negative at six weeks and remained so to the end of the experiment. The tuberculin reactions of the six groups of infected and treated guinea pigs twenty-four and thirty-six weeks after challenge are noted in table 2.

Again, in *Group A* the results indicated the most effective control of the tuberculous infection, 15 animals (25 per cent of the original group) having a negative cutaneous reaction to tuberculin at thirty-six weeks. Eight animals (13 per cent) in each of *Groups C* and *D* were negative to tuberculin, and in *Group E* there were only 4 animals (7 per cent) negative to tuberculin thirty-six weeks after infection. *Groups B* and *F* did not contain a single tuberculin-negative animal thirty-six weeks after infection.

In this study, the two pretreatment *Groups C* and *D* had a slightly larger number of animals whose tuberculin reactions reverted from positive to negative than did *Group E*, in which the animals, with the same infection and treatment, received no isoniazid prior to challenge.

In *Groups A*, *C*, *D*, and *E*, there were more animals tuberculin negative at thirty-six weeks than at twenty-four weeks after infection, even though, in *Groups A*, *C*, and *E*, treatment had been discontinued for at least twenty-four weeks. In no instance did the reaction of an animal which reverted to negative as a result of treatment convert to positive during the period of observation (thirty-six weeks).

The finding that the groups of animals which otherwise achieved the most

TABLE 2

STUDY 2

CUTANEOUS REACTION TO TUBERCULIN OF 6 GROUPS OF INFECTED AND ISONIAZID-TREATED GUINEA PIGS 24 WEEKS AND 36 WEEKS AFTER CHALLENGE

Animal Group	Total Number at Start		Observations at 24 Weeks										Observations at 36 Weeks									
			Died in Interval		Still Alive		Tuberculin Reaction of Surviving Animals						Died in Interval		Still Alive		Tuberculin Reaction of Surviving Animals					
							Strongly Positive		Weakly Positive		Negative						Strongly Positive		Weakly Positive		Negative	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Control No. 3	60	*100*	1	*2*	59	*98*							3	*5*	57	*95*						
Not infected; not treated							0	*0*	0	*0*	59	*98*					0	*0*	0	*0*	57	*95*
Group A	60	*100*	7	*12*	53	*88*							7	*12*	53	*88*						
Pretreated; infected with 2×10^3 units; therapy 6 weeks							34	*57*	8	*13*	11	*18*					33	*55*	5	*8*	15	*25*
Group B	60	*100*	23	*38*	37	*62*							32	*53*	28	*47*						
Pretreated; infected with 3×10^3 units; therapy 6 weeks							32	*53*	5	*8*	0	*0*					25	*42*	3	*5*	0	*0*
Group C	60	*100*	9	*15*	51	*85*							11	*18*	49	*82*						
Pretreated; infected with 3×10^6 units; therapy 12 weeks							36	*60*	9	*15*	6	*10*					34	*57*	7	*12*	8	*13*
Group D	60	*100*	8	*13*	52	*87*							12	*20*	48	*80*						
Pretreated; infected with 3×10^6 units; therapy 50 weeks							35	*58*	10	*17*	7	*12*					31	*52*	9	*15*	8	*13*
Group E	60	*100*	10	*17*	50	*83*							12	*20*	48	*80*						
Infected with 3×10^6 units; therapy for 12 weeks, started simultaneously with infection							39	*65*	9	*15*	2	*3*					35	*58*	9	*15*	4	7
Group F	60	*100*	23	*38*	37	*62*							27	*45*	33	*55*						
Infected with 3×10^6 units; therapy for 12 weeks, started 14 days after infection							35	*58*	2	*3*	0	*0*					29	*48*	4	*7*	0	*0*

effective control of the tuberculous infection also had the lowest degrees of tuberculin sensitivity is in agreement with the observations of the USPHS investigators. The present study, however, did not confirm an increase in size of the tuberculin reactions after discontinuation of isoniazid which the USPHS investigators reported (2).

Study 3—visceral organ cultures for M. tuberculosis: The cultural results of the three control groups and the six infected and treated groups of guinea pigs are found in table 3. Among the six infected and treated groups of animals, the results roughly paralleled those obtained in the survival and tuberculin test studies. Again, the animals in *Group A* seemed to have the most effectively controlled disease, 50 (83 per cent) of the original group having cultures of their organs negative for tubercle bacilli at sacrifice thirty-six weeks after challenge. *Groups C* and *D* did almost as well, 47 animals of each group (78 per cent) having negative cultures thirty-six and fifty weeks, respectively, after infection. *Group B* animals again showed the least effective control of tuberculosis, only 12 (20 per cent) of the original group of animals having cultures negative for *M. tuberculosis* thirty-six weeks after infection. The animals in *Groups E* and *F* showed an intermediate response (66 and 43 per cent, respectively) of the animals at risk having negative cultures at sacrifice.

About 97 per cent of all of the treated animals which died of disease had gross tuberculous lesions of the viscera and smears and cultures positive for *M. tuberculosis*. This was also true of 23 of the 34 sacrificed animals from whose organs tubercle bacilli were cultured (3 animals in *Group A*, 16 animals in *Group B*, and 4 animals in *Group F*). The remaining 11 guinea pigs which were found to harbor tubercle bacilli after sacrifice (3 from *Group C*, one from *Group D*, 4 from *Group E*, and 3 from *Group F*) had no gross tuberculous lesions, negative smears for tubercle bacilli, and one to 8 colonies of *M. tuberculosis* on culture from normal-appearing splenic tissue. All of the animals with organ cultures negative for *M. tuberculosis* also had grossly normal-appearing organs and smears negative for tubercle bacilli.

Of the 246 infected and treated animals surviving thirty-six weeks and culturally analyzed in table 3, 35 (14 per cent) had negative tuberculin reactions, and 211 (86 per cent) had positive tuberculin reactions. Of the 35 tuberculin-negative animals, 22, after sacrifice, had grossly normal organs from which no tubercle bacilli could be demonstrated by smear or culture; the remaining 13 animals, saved for *Study 4*, were assumed to have the same findings. Of the 211 tuberculin-positive animals sacrificed at thirty-six weeks, 34 (16 per cent) had organs positive for tubercle bacilli. Sixteen of the 34 culturally positive animals were found among 28 tuberculin-positive sacrificed guinea pigs in *Group B*, while the remaining 18 culturally positive animals were scattered among the 183 tuberculin-positive sacrificed guinea pigs from the other five treated groups (*See* table 3).

Isoniazid susceptibility and catalase activity of cultures: The isoniazid susceptibility and catalase activity of the cultures of tubercle bacilli isolated from the organs of all of the 126 isoniazid-treated animals with positive cultural findings

TABLE 3

STUDY 3

RESULTS OF ORGAN CULTURES FOR M. TUBERCULOSIS OF 3 CONTROL GROUPS AND 6 INFECTED AND ISONIAZID-TREATED GROUPS OF GUINEA PIGS AT DEATH OR AT SACRIFICE 36 TO 50 WEEKS AFTER CHALLENGE*

Animal Group	Total		Died of Disease				Sacrificed					Tuberculin Negative, Presumed to Have Negative Culture†	Combined Cultural Results					
				Culture for *M. Tuberculosis*				Culture for *M. Tuberculosis*					Positive Culture		Negative Culture		Contaminated Culture	
								Tuberculin Positive		Tuberculin Negative								
	Number	Per Cent	Total Number	Positive Culture	Negative Culture	Contaminated Culture	Total Number	Positive Culture	Negative Culture	Positive Culture	Negative Culture		Number	Per Cent	Number	Per Cent	Number	Per Cent
Control No. 1	60	*100*																
Infected with 2×10^3 units; no therapy			60	60	0	0	0						60	*100*	0	*0*	0	*0*
Control No. 2	60	*100*																
Infected with 3×10^6 units; no therapy			60	60	0	0	0						60	*100*	0	*0*	0	*0*
Control No. 3‡	47	*100*																
Not infected; no therapy			3	0	3	0	44	0	0	0	44		0	*0*	47	*100*	0	*0*
Group A	60	*100*																
Pretreated; infected with 2×10^3 units; therapy for 6 weeks			7	7	0	0	53	3	35	0	15		10	*17*	50	*83*	0	*0*
Group B	60	*100*																
Pretreated; infected with 3×10^6 units; therapy for 6 weeks			32	30	0	2‖	28	16	12	0	0		46	*77*	12	*20*	2‖	*3*
Group C	60	*100*																
Pretreated; infected with 3×10^6 units; therapy for 12 weeks			11	10	1	0	43	3	38	0	2	6	13	*22*	47	*78*	0	*0*
Group D	60	*100*																
Pretreated; infected with 3×10^6 units; therapy for 50 weeks			12	12	0	0	41	1	39	0	1	7	13	*22*	47	*78*	0	*0*

Group E§ Infected with 3 × 10^6 units; therapy for 12 weeks started simultaneously with infection	47	*100*	12	11	0	1	35	4	27	0	4		15	*32*	31	*66*	1	*2*
Group F Infected with 3 × 10^6 units; therapy for 12 weeks started 14 days later	60	*100*	27	22	0	5#	33	7	26	0	0		29	*48*	26	*43*	5#	*8*
All groups of infected and treated animals	347	*100*	101	92	1	8	233	34	177	0	22	13	126	*36*	213	*61*	8	*2*

* All surviving animals were sacrificed 36 weeks after challenge except for those in *Group D* which were sacrificed at 50 weeks.
† Removed from group at 36 weeks for Study 4.
‡ Thirteen tuberculin-negative animals removed for Study 4.
‖ Positive for *M. tuberculosis* on smear.
§ Thirteen healthy tuberculin-positive animals removed for Study 4.
# Two animals had positive smears for *M. tuberculosis*.

TABLE 4

ISONIAZID SUSCEPTIBILITY AND CATALASE ACTIVITY OF TUBERCLE BACILLI ISOLATED BY CULTURE FROM VISCERAL ORGANS OF 126 INFECTED AND ISONIAZID-TREATED GUINEA PIGS AFTER DEATH OR SACRIFICE

Animal Group	Number of Cultures Tested	Catalase Activity		Animals Dead—Time of Death in Weeks												Animals Sacrificed 36 Weeks After Challenge				Total			
				0–6				6–12				12–36											
				Isoniazid Susceptibility Expressed as Highest Concentration of Drug Allowing Any Growth in γ per ml. of Medium																			
		Amount In Per Cent	Number	0	0.1–1	1–5	5–100	0	0.1–1	1–5	5–100	0	0.1–1	1–5	5–100	0	0.1–1	1–5	5–100	0	0.1–1	1–5	5–100
Group A																							
Pre-therapy; infected with 2×10^3; therapy for 6 weeks	10	50–100	9	1				2				3						2	1	6	0	2	1
		25–50	1						1												1		
Group B																							
Pre-therapy; infected with 3×10^6; therapy for 6 weeks	46	50–100	30	2				7				4	4	3		7	1	1	1	20	5	4	1
		25–50	16						2	1			3	2	2		1	2	3		6	5	5
Group C																							
Pre-therapy; infected with 3×10^6; therapy for 12 weeks	13	50–100	9	2				2				1	1			3				8	1		
		25–50	4						1				2		1						3		1
Group D																							
Pre-therapy; infected with 3×10^6; therapy for 50 weeks	13	50–100	9	2				1	2	1		2				1*				6	2	1	
		25–50	4												4							4	
Group E																							
Infected with 3×10^6; therapy was started simultaneously; 12 weeks	15	50–100	11	1				3	1			1	1			4				9	2		
		25–50	4						2	1				1							2	2	

Group F																							
Infected with 3×10^6; therapy was started 14 days later; 12 weeks	29	50–100	25	4				7				8	1			3	1	1		22	2	1	
		25–50	4						1					1			1	1			2	2	
Total	126	50–100	93	12	0	0	0	22	3	1	0	19	7	3	0	18	2	4	2	71	12	8	2
		25–50	33	0	0	0	0	0	7	2	0	0	5	4	7	0	1	4	3	0	13	10	10
Total				12	0	0	0	22	10	3	0	19	12	7	7	18	3	8	5	71	25	18	12

* Sacrificed 50 weeks after challenge.

after death or sacrifice are found in table 4. The isoniazid susceptibility of the cultures is expressed as the highest concentration of the drug in micrograms per milliliter of medium which allowed any growth. The catalase activity is stated in terms of percentage of activity in comparison with a control strain of *M. tuberculosis* which was known to be completely isoniazid susceptible and catalase active (7). In no instance was the catalase activity of a tested culture less than 25 per cent. All of the cultures of *M. tuberculosis* isolated at death from the infected but untreated animals in control *Groups Nos. 1* and *2* were completely catalase active, and 80 per cent of the cultures were also completely isoniazid susceptible; 20 per cent of the cultures contained a very small number of organisms which grew out in medium containing 0.1 γ of isoniazid per ml.

It may be seen in table 4 that all animals which died within the first six weeks following challenge had organisms which were completely isoniazid susceptible and catalase active. Of the 35 animals which died between the sixth and twelfth weeks following infection, 13 (37 per cent) had organisms with some degree of isoniazid resistance *in vitro*. Since the distribution of isoniazid-susceptible and -resistant strains of tubercle bacilli among animals which died between the twelfth and twenty-fourth weeks was essentially the same as among those which died between the twenty-fourth and thirty-sixth weeks, cultures from animals dying between the twelfth and thirty-sixth weeks have been grouped together. Of the 45 animals that died in this interval after challenge, 26 (58 per cent) had organisms with some degree of isoniazid resistance. Of the 34 sacrificed animals with cultures positive for tubercle bacilli, 16 (47 per cent) had isoniazid-resistant organisms. Thus, of the entire group of 126 infected and treated guinea pigs from which tubercle bacilli were isolated, 55 (44 per cent) had organisms with some degree of isoniazid resistance; among the 114 animals which died or were sacrificed six or more weeks after challenge, 55 (almost 50 per cent) had isoniazid-resistant tubercle bacilli.

In general, there seemed to be no outstanding difference in the pattern of emergence of isoniazid-resistant organisms among the six infected and treated groups of animals. There was a tendency for the isolated cultures to show some loss of catalase activity in association with increased loss of isoniazid susceptibility, but there were a few cultures with highly resistant organisms which revealed very little, if any, loss of catalase activity. In this connection it should be emphasized that all of the cultures of tubercle bacilli which showed any isoniazid resistance were invariably made up of mixtures containing organisms of varying degrees of susceptibility to isoniazid; in no instance, either at death or sacrifice, did an organ culture grow a pure isoniazid-resistant strain of *M. tuberculosis*. Some of the cultures, however, contained highly resistant strains; two organ cultures contained tubercle bacilli which grew on medium containing 100 γ of drug per ml.

The tubercle bacilli isolated from *normal-appearing* splenic tissue from treated animals sacrificed at thirty-six weeks[3] after infection (11 animals from *Groups*

[3] One culture was isolated from an animal in *Group D* fifty weeks after infection.

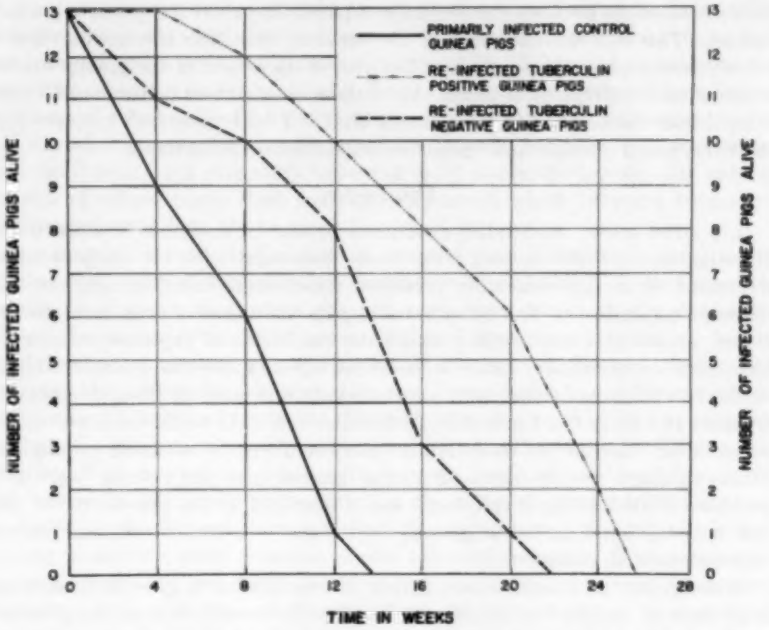

FIG. 2. Chart showing survival following reinfection with 3×10^6 bacterial units of *M. tuberculosis* of 13 tuberculin-positive and 13 tuberculin-negative guinea pigs which survived forty-one weeks after massive primary infection and treatment with isoniazid.

C, *D*, *E*, and *F*) were all completely susceptible to isoniazid and were catalase active, a finding which is in agreement with that of other investigators (4, 8).

Study 4—reinfection of surviving animals: Survival curves for the two groups of massively reinfected animals and the primarily infected control group are shown in figure 2. In the control group, the 13 animals died of their primary tuberculous infection within thirteen weeks, and all had typically extensive tuberculous lesions in the spleen, liver, and lungs, while smears and cultures from all organs revealed large numbers of tubercle bacilli.

In contrast, the two groups of reinfected animals withstood the infection somewhat better. The tuberculin-positive group of animals survived for the longest period, the last animal dying of tuberculosis twenty-seven weeks after reinfection. In this group, an enlarged, grossly involved spleen was the only organ in which visible tuberculous lesions were noted in each of the autopsied guinea pigs except in 5 animals which also had small discrete lesions in the liver. All splenic tissue and five hepatic cultures and smears were positive for tubercle bacilli. The reinfected animals lost weight more gradually than those in the control group.

The tuberculin-negative group of reinfected animals revealed findings which

were intermediate between the control group and the tuberculin-positive group of animals. This was not only true of the survival time (the last animal died at twenty-two weeks) and weight loss, but also of the extent of the grossly evident tuberculous involvement at death. All of these animals had cultures and smears from splenic tissue positive for tubercle bacilli; 7 had "positive" cultures from the liver; and 2 animals had "positive" cultures from lung tissue.

Discussion

In a strict sense, the studies conducted by the U. S. Public Health Service investigators and the present writers are concerned with the early isoniazid treatment of an experimentally produced tuberculous infection, and not with chemoprophylaxis (3). The latter term implies the use of a drug to *prevent infection*, an obvious impossibility under the conditions of experiments in which large numbers of virulent tubercle bacilli are injected into the animals. Animals exposed to tubercle bacilli under natural conditions, such as those described by Schmidt (4), Riley (9), Lurie (10), or Middlebrook (11) would be more suitable subjects for experiments to determine the usefulness of isoniazid prophylaxis. Thus, although the present and similar studies may have some bearing on problems related to the prophylactic use of isoniazid, in the present report they will be considered as investigations in the early treatment of experimental tuberculosis with isoniazid.

In designing the present investigation, it was decided to give the guinea pigs large doses of isoniazid by injection rather than by mouth, thus allowing them to eat an unrestricted healthful diet, instead of the dehydrated pellets fortified with Vitamin C which had to be given by the U. S. Public Health Service group of investigators in order to force the animals to drink their isoniazid-containing water (1, 2). Guinea pigs ordinarily do not drink water, but obtain their fluids from fresh, leafy green vegetables, and the alteration of their diet so as to deprive them of this very important constituent may have contributed to the extremely high mortality rate (approximately 40 per cent in forty weeks) observed in the healthy normal controls in the USPHS studies. Moreover, the present administration of 5 mg. per 100 gm. of isoniazid by injection *assured* sufficient isoniazid for every animal treated without exception. There were no noticeable deleterious effects from either the triweekly intraperitoneal injections or the relatively huge doses of isoniazid employed.

The early use of isoniazid for a tuberculous infection to prevent the development of serious tuberculous disease in the future rests on two premises advanced by Palmer and his colleagues (2): (*a*) Early isoniazid treatment can apparently convert a massive inoculation of virulent tubercle bacilli into a controlled, benign, tuberculous infection in guinea pigs; and (*b*) such animals acquire some resistance against subsequent reinfection after the drug has been discontinued. It is implied by these investigators that the size of the infecting dose may not be of too much significance in determining the ultimate outcome of early isoniazid treatment in that "multiplication of bacilli *in vivo* may well convert a small initial inoculum into a massive infection within a short period of time."

In sharp contrast to this point of view is the opinion of Dubos (3) and others

(4, 8) that effective early isoniazid administration would significantly interfere with the development of specific immunity if given at a time when the animal was infected with very small numbers of organisms. This would probably be the prevailing situation in humans, who are always infected with relatively small numbers of tubercle bacilli even under conditions of heavy exposure. On the other hand, it is argued that, with a sufficiently large inoculum, it is possible for an animal to acquire immunity following early isoniazid therapy, but only at the risk of future reactivation from tubercle bacilli which have not been completely eradicated from the tissues.

Fortunately, several studies now in progress should eventually resolve this difference of opinion. Investigations carried out in monkeys (4) and mice (5) already have shown that, when given in large doses, isoniazid can profoundly inhibit the antituberculous immunity induced by primary infection due to *small numbers* of virulent tubercle bacilli. The present studies also confirm the fact that the size of the infecting dose has a very important influence on the results of early isoniazid treatment. The animal group infected with the smaller number of organisms (*Group A*) achieved the best control over the tuberculous infection. This was accomplished with only six weeks of treatment, compared with twelve to fifty weeks of treatment in the other effectively treated groups. It should be pointed out, however, that this group also contained the largest number of animals with reversion of the tuberculin reaction from positive to negative, indicating not only loss of allergy, but also some loss of acquired immunity. Thus, the more effectively the tuberculous infection was controlled, the greater was the tendency for isoniazid therapy to nullify the antigenic effects of that infection.

It was also demonstrated in the present studies that, in addition to the size of the challenge inoculum, both the time at which treatment is started in relation to time of infection and the duration of treatment have a considerable influence on the effectiveness of early isoniazid therapy. Pretreatment seemed to offer very little advantage over starting treatment simultaneously with challenge, but a delay of fourteen days after infection resulted in unsatisfactory control of the infection. Similarly, whereas six weeks of treatment seemed to be sufficient for almost maximal control of the tuberculous infection in the animals inoculated with the smaller dose of tubercle bacilli, it was found insufficient for adequate control of the infection caused by the larger inoculum. However, isoniazid treatment for twelve weeks achieved very good control of the massive infection, which was not improved by extending the duration of treatment to fifty weeks. Of special interest is the observation that one animal treated for fifty weeks still had demonstrable tubercle bacilli in the spleen.

The results of treatment in *Groups F* and *B* compared with those of the other groups indicate that in the massively infected animals the disadvantage of starting therapy fourteen days after infection could be overcome to some extent by administering isoniazid for an adequate period (twelve weeks), while the advantage of starting therapy simultaneously with infection could be dissipated by giving the drug for too short a period (six weeks).

Of some interest are the variations in the state of the tuberculous disease and

the skin sensitivity to tuberculin among the 246 surviving infected and treated animals analyzed (table 3). Of the 211 survivors with positive tuberculin reactions at the time of sacrifice, 23 had gross morphologic and bacteriologic evidence of tuberculous disease; 11 had normal-appearing organs, but demonstrable tubercle bacilli in the spleen; and 177 had grossly normal organs from which tubercle bacilli could not be isolated. The remaining 35 animals had negative tuberculin reactions and, in the 22 of these sacrificed, grossly normal organs were found from which tubercle bacilli could not be demonstrated.

Thus, in the majority of the survivors (72 per cent), the acquired immunity was probably maintained despite normal-appearing organs which were free of organisms as far as could be determined by present-day methods. Only by longer observation of a similar group of animals would it be possible to ascertain whether or not some of these animals harbored a "latent" infection, characterized by very late reactivation of disease, such as was demonstrated in mice by McDermott and associates (8), in monkeys by Schmidt (4), and in guinea pigs by Sher and co-workers (6). In the other positive tuberculin-reacting survivors (14 per cent), although acquired immunity was maintained, it is quite likely that, in view of the readily demonstrable tubercle bacilli in their organs, a longer period of observation might have revealed a late reactivation of the residual "arrested" tuberculosis (6). As for the tuberculin-negative survivors (14 per cent), while they were probably free of the danger of late reactivation, it is possible that with a longer period of observation they might have eventually lost more of their acquired immunity which, thirty-six weeks after infection, already showed an appreciable decline. It is of interest that a negative tuberculin reaction was maintained for at least twenty-four weeks after discontinuation of treatment in 26 of the infected and treated animals. The presence of some acquired immunity in 13 of these animals challenged with a second inoculum of tubercle bacilli is in accordance with the observations of other investigators that the loss of skin allergy does not necessarily indicate complete loss of acquired immunity.

Particular attention should be given to the fact that 44 per cent of the 126 tested cultures of tubercle bacilli isolated from the organs of the same number of infected and treated animals, irrespective of group, were isoniazid-resistant to some degree, and completely or partially catalase active. To what extent a longer period of treatment might have affected the emergence of resistant organisms in the animals infected with the smaller inoculum (2×10^3 bacterial units) was, unfortunately, not determined. Moreover, it remains to be determined to what extent emergence of drug-resistant tubercle bacilli would be a problem in animals adequately treated after challenge with a *very small* number of organisms. It should be noted that in the three groups of animals massively infected but treated *promptly* for at least *twelve weeks* (*Groups C*, *D*, and *E*) drug-resistant organisms were not found among any of the surviving animals.

* * *

The present findings indicate that, under the experimental conditions described, very early administration of enormous doses of isoniazid cannot be depended upon to eradicate a tuberculous infection, to prevent the rapid emer-

gence of drug-resistant tubercle bacilli, or safely to permit the development and the maintenance of a high degree of acquired immunity in guinea pigs. On the basis of these findings, therefore, the present writers are of the opinion that the widespread administration of isoniazid to population groups at a low risk of developing significant tuberculosis is not justified without a more careful appraisal of the potential hazards of such a practice.

Summary

Six groups of 60 guinea pigs each were infected with virulent tubercle bacilli and were started on intensive isoniazid treatment either at the time of challenge or soon thereafter under various experimental conditions.

Under the conditions of the present studies, isoniazid did not prevent the development of tuberculous infection, but was highly effective in controlling it. The results of early isoniazid treatment were profoundly influenced by the size of the infecting inoculum, the tuberculous infection being most effectively controlled in the group of animals which received the smaller of the two inocula used. Pretreatment had no appreciable advantage over treatment started simultaneously with infection. A delay of fourteen days in starting treatment, however, had a decidedly unfavorable effect. In the animals massively inoculated, treatment for six weeks was definitely inferior to treatment for twelve weeks; treatment continued for fifty weeks had no outstanding advantage over that given for only twelve weeks.

Of 246 treated animals which were alive thirty-six weeks after primary infection, 35 (14 per cent) had negative tuberculin skin reactions at the time of sacrifice. Among the 211 surviving animals with persistently positive tuberculin reactions (86 per cent), 34 (14 per cent) had culturable tubercle bacilli in their organs after sacrifice.

Of 126 infected and treated guinea pigs from which tubercle bacilli were isolated after death or sacrifice, 55 (44 per cent) had organisms with some degree of isoniazid resistance.

Treated guinea pigs which survived a first infection demonstrated an increased immunity to a second challenge. The animals which were tuberculin positive at the time of reinfection showed appreciably more immunity than those tuberculin negative.

Sumario

Los Efectos de la Isoniacidoterapia Temprana en la Tuberculosis Experimental del Cobayo

A seis grupos, compuestos de 60 cobayos cada uno, se les inyectaron bacilos tuberculosos virulentos, iniciándose en ellos tratamiento intenso con isoniacida, ya en el momento de la provocación o poco después en condiciones variables de experimentación.

En las condiciones de los estudios actuales, la isoniacida no impidió la aparición de infección tuberculosa, pero se mostró sumamente eficaz en su dominio. Los resultados de la isoniacidoterapia temprana se vieron profundamente afectados por el tamaño del inóculo infectante, dominándose más eficazmente la infección tuberculosa en el grupo de animales que recibió el más pequeño de los dos inóculos usados. El pretratamiento no mostró ninguna ventaja apreciable sobre el tratamiento iniciado simultáneamente con la inyección. Sin embargo, una demora de catorce días en la iniciación del tratamiento ejerció un influjo

decididamente desfavorable. En los animales inoculados masivamente, el tratamiento durante seis semanas fué positivamente inferior al de doce semanas; el tratamiento continuo por cincuenta semanas no mostró mayor ventaja sobre el administrado únicamente doce semanas.

De 246 animales que se hallaban vivos a los treinta y seis semanas de la infección primaria, 35 (14 por ciento) acusaron cutirreacciones negativas a la tuberculina en la fecha en que fueron sacrificados. Entre los 211 animales sobrevivientes (86 por ciento) que tenían reacciones persistentemente positivas a la tuberculina, 34 (14 por ciento) tenían bacilos tuberculosos cultivables en sus órganos después de sacrificarlos.

De 126 cobayos infectados y tratados de los se que se aislaron bacilos tuberculosos después de la muerte o del sacrificio, 55 (44 por ciento) tenían microbios dotados de alguna resistencia a la isoniacida.

Los cobayos tratados que sobrevivieron la primera infección mostraron mayor inmunidad a la segunda provocación. Los animales que eran tuberculinopositivos en la fecha de la reinfección revelaron inmunidad apreciablemente mayor que los tuberculinonegativos.

Resume

Les effets de l'isoniazido-thérapie précoce dans la tuberculose expérimentale du cobaye

Six groupes de 60 cobayes chacun furent inoculés avec des bacilles tuberculeux virulents et ont été mis à l'isoniazido-thérapie intensive, soit au moment de l'inoculation soit aussitôt après, dans des conditions expérimentales variées.

Dans les conditions de l'étude actuelle, l'isoniazide ne prévenait pas le développement de l'infection tuberculeuse, mais s'est montrée extrêmement efficace pour la combattre. Les résultats du traitement précoce par l'isoniazide étaient profondément influencés par la taille de l'inoculum infectant, l'infection tuberculeuse étant le plus efficacement contrôlée dans le groupe d'animaux qui recevaient le plus réduit des deux inoculums utilisés. Un traitement antérieur ne présentait pas d'avantage appréciable sur le traitement entrepris simultanément avec l'injection. Un délai de quatorze jours dans le début du traitement avait toutefois un effet nettement défavorable. Chez les animaux inoculés de façon massive, le traitement pendant six semaines était nettement inférieur au traitement d'une durée de douze semaines; le traitement poursuivi pendant cinquante semaines n'offrait pas d'avantage spécial sur celui administré pendant douze semaines seulement.

Sur les 246 animaux traités qui survivaient trente-six semaines après la primo-infection, 35 (14%) avaient des réactions cutanées négatives à la tuberculine au moment où l'animal était sacrifié. Parmi les 211 animaux survivants (86%) ayant des réactions positives persistantes à la tuberculine, 34 (14%) avaient des bacilles tuberculeux cultivables dans leurs organes après avoir été sacrifiés.

Sur les 126 cobayes infectés et traités chez lesquels les bacilles tuberculeux ont été isolés après qu'ils soient morts ou sacrifiés, 55 (44%) avaient des bacilles possédant un certain degré de résistance à l'isoniazide.

Les cobayes traités qui survivaient à l'infection initiale présentaient une immunité croissante à une deuxième inoculation. Les animaux qui étaient "positifs" à la tuberculine au moment de la réinfection présentaient une immunité nettement plus élevée que ceux ayant une réaction à la tuberculine négative.

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TUBERCLE BACILLUS WAX AS AN EXPERIMENTAL VACCINE AGAINST MOUSE TUBERCULOSIS[1, 2]

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INTRODUCTION

Purified tubercle bacillus wax[3] (1) has proved effective as an experimental antigenic adjuvant (2). The present writers have reported a significant immunogenic enhancement of heat-killed BCG by *n*-hexadecane,[4] although hexadecane alone did not affect the resistance of mice to experimental tuberculosis (3). During studies of a tubercle bacillus wax preparation as a proposed adjuvant to BCG, however, it was shown that wax plus hexadecane could significantly immunize mice in the absence of BCG. The wax preparation without hexadecane was less effective.

METHODS

Most of the experimental procedures have been described elsewhere (3, 4). Purified tubercle bacillus wax (1) is highly soluble in chloroform and moderately soluble in hexadecane at temperatures of more than 30°C. A fine but temporary aqueous suspension of wax plus hexadecane was prepared by dissolving wax in hexadecane, adding this solution to vials of distilled water, and shaking hard by hand. To prepare a suspension of "wax only" in water, wax was dissolved in small volumes of chloroform, added to vials of slightly warm water, and shaken. These vials were then unstoppered and the chloroform was allowed to evaporate. Residual chloroform in such suspensions of "wax only" produced no noticeable anesthetic effect on mice.

Mice were vaccinated by the subcutaneous and intraperitoneal routes, respectively. The subcutaneous dose of wax was generally 0.2 mg. in 1 ml. of either water or 1 per cent hexadecane, and 2 injections of this dose were usually given fourteen days apart. Methods using BCG and the intraperitoneal route have been described elsewhere (3). All BCG used in these experiments was heat killed (70°C.—1 hour). Intravenous challenge with 1 mg. of H37Rv followed the last vaccine injection (with one exception) by two or more weeks. Most mice were at least two weeks older at the time of challenge than those described before (3, 4), due mainly to this schedule of two vaccine injections.

Control mice were untreated save in one experiment in which a new brand of highly purified hexadecane was introduced. Here two groups of control mice, injected with different brands of hexadecane (in water) were compared with an untreated group. The three

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[3] Dr. Esmond R. Long kindly supplied 5 gm. of purified tubercle bacillus wax from the Henry Phipps Institute, Philadelphia 47, Pennsylvania. This wax was an original preparation by Dr. R. J. Anderson.

[4] Grateful acknowledgment is made for donations of purified *n*-hexadecane and other hydrocarbons by Mr. Bingham J. Humphrey, Humphrey-Wilkinson, Inc., De Vine Street, North Haven, Connecticut.

groups gave similar results, confirming previous findings on the noneffectiveness of hexadecane alone (3).

Mouse groups were compared for differences between mean fatality times (in days) and final survival (4, 5) rates (per cent surviving). When the numbers of mice were small and/or n was less than $10/p$, probabilities were calculated from the binomial distribution with the aid of factorials (6). "Survivors" were mice living when experiments were terminated, usually six weeks after challenge. Autopsies demonstrated that tuberculosis might have killed some "survivors" had the experiments been continued longer. Intergroup comparisons were made within sexes, and sex differences were studied within groups. It must be emphasized that all means are geometric means.

Results

Tests of intraperitoneal vaccination are summarized in table 1. Each vaccinated group shows significantly ($p=0.05$ or less) more immunity than its controls for at least one sex and by one method of comparison. These differences occurred mostly in males (4).

The following experiments concern subcutaneous vaccination only. Most of the mice were two or more weeks older than those noted in table 1. Mice vaccinated subcutaneously with wax plus hexadecane were compared with controls in twelve experiments; other comparisons involved fewer protocols. These tests are summarized in table 2. The group vaccinated with wax only was the only group that failed to show significantly more immunity than its controls for both sexes and by at least one method of comparison.

The purified tubercle bacillus wax preparation supplied to the writers contains

TABLE 1

Intraperitoneal Vaccination Experiments

Experimental Groups	Number of Mice		Mean Fatality Time (days)		Per Cent Surviving		Significant p Values (0.05 or less)* Between Experimental and Control Groups			
							Fatality Times		Per Cent Surviving	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Wax plus hexadecane	32	35	25.2	17.4	41	20	<0.001	—	<0.001	—
Controls	54	47	17.3	15.4	6	9				
BCG plus hexadecane	12	11	26.9	22.6	58	36	—	0.05	<0.01	—
BCG only	13	11	21.3	19.6	38	9	—	—	0.02	—
Wax plus hexadecane	13	11	33.3	20.4	46	18	<0.02	<0.01	<0.01	—
Controls	13	13	21.7	17.4	0	8				
Wax plus hexadecane	19	24	21.3	16.1	37	21	<0.001	—	0.01	—
Wax only	11	13	19.5	16.4	9	0	0.05	—	—	—
Controls	41	34	16.0	14.8	7	9				

* Additional significant differences are as follows:
First section: Fatality times, both groups ♂ > ♀.
Second section: Fatality times, wax + hexadecane and controls ♂ > ♀.
Third section: Fatality times, wax + hexadecane and controls ♂ > ♀.
Per cent surviving, wax + hexadecane ♂ > ♀.

TABLE 2

SUBCUTANEOUS VACCINATION EXPERIMENTS

Experimental Groups	Number of Mice		Mean Fatality Time (days)		Per Cent Surviving		Significant p Values (0.05 or less)* Between Experimental and Control Groups			
							Fatality Times		Per Cent Surviving	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Wax plus hexadecane	331	346	20.8	18.9	30.2	31.8	<0.001	<0.001	<0.001	<0.001
Controls	212	208	17.5	16.3	9.9	7.7				
Wax plus hexadecane	70	68	21.6	18.5	24	31	<0.001	—	—	<0.001
BCG plus hexadecane	67	71	20.1	17.6	24	27	<0.01	—	—	<0.01
Controls	73	74	17.3	17.2	15	8				
Wax plus hexadecane	42	46	22.0	18.7	33	37	<0.001	—	—	<0.01
Wax only	45	47	17.5	16.8	9	13	—	—	—	—
Controls	31	22	16.4	15.8	19	4				

* Additional significant differences are as follows:

First section: Fatality times, both groups ♂ > ♀.

Second section: Fatality times, wax + hexadecane ♂ > ♀. Fatality times, BCG + hexadecane ♂ > ♀.

Third section: Both comparative methods, wax + hexadecane ♂ > wax only ♂.

Both comparative methods, wax + hexadecane ♀ > wax only ♀. Fatality times, wax + hexadecane ♂ > ♀.

myriads of mycobacteria that are almost completely nonacid-fast. In order to demonstrate most of these organisms, it was necessary to decolorize Ziehl-Neelsen stains briefly with alcohol only. They then appeared as thin and often angled tubercle bacilli, many of which contained typical granules.

The following experiment assayed the immunizing potency of wax after almost all organisms had been removed by ultracentrifugation:

Wax was dissolved in purified *n*-octane (which is relatively innocuous to tubercle bacilli and has a low specific gravity) to a concentration of 20 mg. per ml. This solution was centrifuged at room temperature for one and one-half hours at 35,000 r.p.m. The top three-fourths of the liquid was then removed, diluted with more octane to its original volume, and recentrifuged at the same speed for two and one-half hours, and the whole process was repeated for a third time. The lower fourth of the first centrifugation, which contained wax plus many bacterial bodies and is defined as "first sediment wax," and the supernate of the third centrifugation were left at room temperature. The octane evaporated from them quite rapidly. The original solution of wax in octane was smeared and stained, and showed approximately 40 bacteria per oil immersion field, while wax from the third supernate showed only 2 organisms per 100 fields when similarly examined.

One group of mice was vaccinated subcutaneously with whole (uncentrifuged) wax, a second group received "first sediment wax," and a third group was given wax from the third supernate. Doses were 0.2 mg. of wax in 1 ml. of 1 per cent hexadecane, 2 injections

TABLE 3

ULTRACENTRIFUGATION EXPERIMENT

Experimental Groups	Number of Mice		Mean Fatality Time (days)		Per Cent Surviving		Significant p Values (0.05 or less)* Between Experimental and Control Groups			
							Fatality Times		Per Cent Surviving	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
(*A*) Whole wax plus hexadecane	18	18	21.1	20.1	22	28	0.01	<0.01	—	—
(*B*) First sediment wax plus hexadecane	16	21	19.4	19.6	19	24	0.01	<0.001	—	—
(*C*) Third supernate wax plus hexadecane	18	18	15.7	15.2	0	0	—	—	—	—
(*D*) Controls	15	18	15.8	15.1	0	6				
(*A*) plus (*B*)	34	39	20.3	19.8	21	26	<0.001	<0.001	<0.01	<0.01
(*C*) plus (*D*)	33	36	15.7	15.1	0	3				

* Additional significant differences are as follows:

First section: Fatality times, whole wax + hexadecane ♂ > third supernate wax + hexadecane ♂.

Fatality times, first sediment wax + hexadecane ♂ > third supernate wax + hexadecane ♂.

Both comparative methods, whole wax + hexadecane ♀ > third supernate wax + hexadecane ♀.

Both comparative methods, first sediment wax + hexadecane ♀ > third supernate wax + hexadecane ♀.

being given two weeks apart. All mice (including untreated controls of the same age) were challenged intravenously with 1 mg. of H37Rv two weeks after the last injection.

This experiment is summarized in table 3. The original whole wax and the first sediment wax, each containing many solvent-extracted tubercle bacilli, gave significant protection to both sexes as measured by fatality times, but failed to reach levels of statistical significance with respect to survival. The third supernate wax, containing very few bacilli, completely failed to protect. Because there are no significant differences between whole wax and first-sediment wax and also none between the third supernate wax and controls, it is permissible to pool the similar pairs and compare the two pooled groups, which has been done in the lower part of table 3. With larger groups, the difference between immunized mice (*A* plus *B*) and nonimmune mice (*C* plus *D*) shows increased significance, which is now also demonstrable by the "per cent surviving" method of evaluation.

One experiment compared the protection given by a single large dose of wax plus hexadecane with that conferred by six small doses of the same antigen.

One mouse group received a subcutaneous injection of 0.4 mg. of wax in 1 ml. of 2 per cent hexadecane four weeks before challenge. A second group received the same dose two weeks later. A third group was given an injection containing 0.05 mg. of wax in 1 ml. of $\frac{1}{4}$ per cent hexadecane every other day, starting four weeks before challenge. The plan was

TABLE 4

IMMUNIZATION SCHEDULE EXPERIMENT

Experimental Groups	Number of Mice		Mean Fatality Time (days)		Per Cent Surviving		Significant p Values (0.05 or less)* Between Experimental and Control Groups			
							Fatality Times		Per Cent Surviving	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0.4 mg. wax plus hexadecane, 2 weeks before challenge	14	14	19.4	19.0	57	7	—	0.05	<0.01	—
0.4 mg. wax plus hexadecane, 4 weeks before challenge	15	15	22.3	20.0	27	33	0.02	0.02	—	—
6 injections, each 0.05 mg. wax plus hexadecane from 4 weeks to 18 days before challenge	15	15	22.2	23.2	53	47	0.02	<0.01	0.01	0.02
Controls	14	14	18.0	16.2	7	7				

* Additional significant differences are as follows:
Per cent surviving, 6 injections of wax + hexadecane ♀ > 1 injection of wax + hexadecane ♀ 2 weeks before challenge.
Per cent surviving, 1 injection of wax + hexadecane 2 weeks before challenge ♂ > ♀.
Fatality times, controls ♂ > ♀.

to give these mice eight injections, which would have been the same amount of antigen received by the first two groups; but this plan proved impracticable.

A single subcutaneous injection of wax plus hexadecane causes a small area of local inflammation which often resorbs but sometimes progresses to abscess formation. A second similar injection intensifies this picture. The mice in the third group developed many abscesses and lost weight, and the injections were terminated after the sixth had been given (eighteen days before challenge). This mouse group still did not look well when challenged.

The results are summarized in table 4. Immunized mice of both sexes show significantly more immunity than their controls. The group which received six injections received less antigen than the other immunized mice, but attained a significantly higher resistance level for females as measured by survivals than did the first group, which received one injection. Mice can undoubtedly be sensitized by tubercle bacilli, as demonstrated especially by Gray and Jennings (7). If the mice which had six injections had indeed become highly sensitized, such sensitivity did not inhibit a good degree of immunity.

The effect of subcutaneous wax plus hexadecane was titrated. All vaccinated mice received two injections two weeks apart and were challenged two weeks after the second dose. The largest amount of antigen was 0.2 mg. of wax in 1 ml. of 1 per cent hexadecane. This was reduced by twofold dilutions to 0.0125 mg. of wax in 0.0625 per cent hexadecane, and the mice were immunized with all five doses. All amounts produced apparent protection which failed of significance in only the fourth dilution (0.025 mg. of wax in 1 ml. of 0.125 per cent hexadecane).

In one experiment, mice were vaccinated by two subcutaneous injections of

autoclaved wax plus hexadecane (121°C.—15 minutes) and compared with mice that had received like but unheated antigen and with controls. Both of the wax plus hexadecane groups developed significant immunity relative to the controls. The apparently superior protection produced by the unheated mixture with respect to the autoclaved antigen was found to be not significant.

In one test, mice were vaccinated subcutaneously with a single large dose of 0.75 mg. of wax in 1 ml. of 2 per cent hexadecane. They were challenged two months later and showed a significant level of immunity. In another experiment mice were held for fifteen weeks after a single large wax and hexadecane injection before being challenged. They survived longer than their controls, but these differences were not significant.

Discussion

Wax without hexadecane proved inferior as an immunizing agent. This is analogous to the findings of Raffel and associates (8*a*) concerning the adjuvant action of wax. These investigators showed that egg albumin plus wax in an oil-in-water emulsion produced higher guinea pig serum titers than egg albumin plus wax without oil.

A stable product such as tubercle bacillus wax containing solvent-extracted mycobacteria is an interesting immunizing agent. Raffel (8*b*) has clearly shown that such wax induces sensitivity to tuberculin in guinea pigs and that tuberculin sensitivity does not develop when both tubercle bacilli and residual tuberculoprotein have been removed.

When tubercle bacilli are extracted with solvents and the extracts filtered, it is logical to expect that some bacterial bodies will pass into these extracts. Boissevain and Ryder (9) and Sabin and Doan (10) recognized this at an early stage, and Raffel (8 *b*) described the laborious procedures necessary to rid wax of such organisms. Nevertheless, the present writers were surprised to find what really huge numbers of solvent-extracted tubercle bacilli the wax contained. It is obvious that this wax plus hexadecane can immunize mice significantly against tuberculosis. High-speed centrifugation, as here reported, removed almost all of the bacteria and destroyed this immunizing power.

Other workers have reported that extracted tubercle bacillus fractions can immunize animals. Smith, Grover, and Nungester (11) separated several lipid types of antigen with which they successfully immunized guinea pigs and rats against tuberculosis. Another fraction appeared to lower the resistance of guinea pigs. Weiss and Dubos (12) obtained promising results by immunizing mice with a fraction extracted by hot methyl alcohol from phenol-killed tubercle bacilli. Dubos, Schaefer, and Pierce (13) extracted active material by grinding killed mycobacteria in 88 per cent phenol.

The possibility of killing tubercle bacilli by the first step in a series of mild extractions, and testing the antigenicity of such killed organisms (both with and without adjuvants) following each extractive procedure, particularly interests the present investigators. Smithburn (14) tested the immunity of rabbits that had been vaccinated with partially defatted, killed mycobacteria. Unfortunately,

too few animals were used for conclusive results, and what might have been a good lead was apparently not followed up. Youmans, Millman, and Youmans (15) prepared suspensions of enzymatically active, nonacid-fast particles from H37Ra by grinding and ultracentrifugation. These particles (possessing many characteristics of mitochondria) produced immunity in mice following intraperitoneal injection. The relatively small numbers of live H37Ra which went into these suspensions proved insufficient to engender immunity by themselves.

In experiments here reported, equal amounts of wax plus hexadecane and BCG plus hexadecane engendered similar resistance levels in mice. Both antigens were effective by either the intraperitoneal or the subcutaneous route. It is possible, however, that wax and BCG may stimulate the immune mechanism differently. Work in progress indicates that heated BCG plus hexadecane can stimulate the production of rather low but demonstrable mouse serum agglutinin titers against a heated H37Ra antigen, while wax plus hexadecane has consistently failed to do this.

Previous findings on sex differences of normal and immunized mice in resistance to tuberculosis (4) are verified by the present report on intraperitoneal vaccination. The subcutaneously immunized mice were older. In the subcutaneous series, male and female controls showed differences in response to challenge that were similar to those previously described. Immunized males also lived longer than females, which confirms earlier observations. In this subcutaneous series, however, there were more (though not significantly more) vaccinated female survivors than males. This discrepancy is not yet fully understood.

Tubercle bacillus wax is stable. It can be stored unchanged for long periods at room temperature and its antigenicity is only partly, if at all, degraded by autoclaving. Methods of modifying the preparation of wax in the hope of increasing its antigenicity are being considered.

Summary

Purified tubercle bacillus wax (Anderson), when dissolved in *n*-hexadecane and suspended in water, is a good immunizing agent against experimental tuberculosis in mice. It is effective following either subcutaneous or intraperitoneal injection.

Purified tubercle bacillus wax (Anderson) contains many solvent-extracted tubercle bacilli. This wax lost its immunizing power when most of these bacilli were removed by ultracentrifugation.

Wax is a stable antigen and retains considerable immunizing potency after autoclaving. The immunizing powers of wax plus hexadecane and heat-killed BCG plus hexadecane are similar.

Sumario

La Cera del Bacilo Tuberculoso como Vacuna Experimental contra la Tuberculosis Murina

Disuelta en *n*-hexadecana y suspendida en agua, la cera purificada del bacilo tuberculoso es un buen agente inmunizante contra la tuberculosis experimental en los ratones, resultando eficaz en inyecciones ya subcutáneas o intraperitoneales.

La cera purificada del bacilo tuberculoso (Anderson) contiene muchos bacilos tuberculosos extraídos con solventes. Esta cera perdió su facultad inmunizante al eliminarse la mayor paete de estos bacilos. La cera es un antígeno estable y retiene considerable potencia inmunizante después de ser tratada al autoclave. Las facultades inmunizantes de la cera más hexadecana y del BCG matado al calor más hexadecana son semejantes.

Resume

La cire du bacille tuberculeux comme vaccin expérimental contre la tuberculose de la souris

La cire de bacille tuberculeux purifiée (Anderson), dissoute dans le n-hexadécane et en suspension dans l'eau, est un bon agent d'immunisation contre la tuberculose expérimentale de la souris. Elle est efficace après l'injection sous-cutanée ou intrapéritonéale.

La cire de bacille tuberculeux purifiée (Anderson) renferme de nombreux bacilles tuberculeux extraits par solvants. Cette cire perdait son pouvoir immunisant quand la plupart de ces bacilles étaient extraits. La cire est un antigène stable et elle conserve un pouvoir immunisant considérable après son passage à l'autoclave. La cire plus l'hexadécane et le BCG tué par la chaleur ont un pouvoir immunisant semblable.

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BRONCHIECTASIS AND ACUTE PNEUMONIA[1]

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INTRODUCTION

Bronchiectasis has been considered a disease that originates in childhood after a severe respiratory infection. During the past ten years, however, studies on young adults who recently had bouts of acute pneumonia suggested that adult infections might also be a cause of bronchiectasis (1, 2). Studies of the stability of such "postpneumonic bronchiectasis" have indicated that in some instances the bronchogram naturally reverts to normal (3). The present study is concerned with the incidence, diagnostic features, and stability of bronchiectasis first discovered after recent pneumonic infections. The question of the etiologic role of the immediately preceding pneumonia is discussed.

METHODS

The clinical records and roentgenograms of 94 patients on whom bronchograms were performed over a twenty-eight month period at the U. S. Army Hospital, Fort Dix, New Jersey, were reviewed. The first group consisted of 69 patients selected for bronchography from a total of 1,711 patients seen with acute pneumonia. All but one of the 69 were between eighteen and twenty-five years of age. The second group consisted of 25 patients whose history or chest roentgenograms suggested chronic bronchiectasis. One third of this group were more than thirty years of age and none of these patients had a recent pneumonic process. The entire group of patients were seen by the writers or their colleagues on the Medical Service, and patient selection for bronchography was done on the basis of uniform criteria.

Bronchography was performed on the patients with pneumonia not less than one month after all clinical evidence of activity had subsided, and only after any residual roentgenographic abnormality was shown to be stable for at least three weeks. Repeat studies were made in twenty-four instances and each was performed at least eight weeks after the preceding one. All of the patients were bronchoscoped immediately prior to the first bronchogram. Fifteen milliliters of iodized oil (Lipiodol®) were instilled through an endotracheal catheter into each lung, and postero-anterior and right and left anterior oblique roentgenograms of the chest were made. An attempt was made to visualize the bronchi of all lobes regardless of the extent of the originally suspicious area of lung. Bronchiectasis was diagnosed only by the presence of obvious cylindrical or saccular dilatation of the bronchial lumen. No untoward reaction was encountered in this series except in patients with bronchial asthma. The latter all developed moderately severe wheezing that responded satisfactorily to standard therapy. The iodized oil remained in the lung for a variable period of time. If there was extensive alveolar filling at the time of the first study, then the dye remained detectable for months, although never in sufficient quantity to interfere with a repeat study. If, on the other hand, the dye originally filled only the bronchial tree, then it tended to disappear more rapidly. All patients remained in the hospital for twenty-four hours following the procedure and performed postural drainage. There was no indication that the latter procedure influenced the degree to which the opaque medium was retained.

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Results

Of the 69 patients who recently had acute pneumonia, 29 were found to have bronchiectasis and 39 were found to be normal. One patient had an abnormal bronchogram that reverted to normal on the repeat study. Of the 18 patients who had not had an immediately preceding pneumonia, 5 had bronchiectasis and 13 were normal. Seven patients with asthma and chronic cough were studied. Six were found normal and one patient had an abnormal bronchogram (table 1). Each group will be considered separately.

The 29 patients with recent acute pneumonia who had bronchiectasis represent 1.7 per cent of the total number of patients with pneumonia seen during the same period. There was no significant difference in the past respiratory history of the bronchiectatic subjects compared with the group found to have normal bronchograms. Both groups had a similar incidence of pneumonia, pertussis, and chronic cough (table 2). There were significant differences, however, in the nature of the immediately preceding pneumonia in the two groups. In the bronchiectatic group the mean duration of roentgenographic evidence of pneumonia was two months, while it was one month in the normal group. The difference is even greater when it is considered that the duration of pneumonia was the main indication for performing the bronchogram. Thus, the two months of the bronchiectatic group might be compared more accurately with an average duration of sixteen days found in a group of 100 consecutive pneumonia patients.

TABLE 1

Results of Bronchograms in the Groups Studied

Patient Group	Number	Results		
		Bronchiectasis	Normal	Reversal
Recent pneumonia	69	29	39	1
Chronic respiratory history	18	5	13	—
Asthma	7	1	6	—
Totals	94	35	58	1

TABLE 2

Respiratory History in the Groups Studied

	Pneumonia	Pertussis	Chronic Cough
	per cent	*per cent*	*per cent*
Recent pneumonia			
Bronchiectasis	43	60	31
No bronchiectasis	41	67	36
Total			
Chronic respiratory history			
Bronchiectasis	60	100	80
No bronchiectasis	46	100	85

TABLE 3

COMPARISON OF IMMEDIATELY PRECEDING PNEUMONIA IN BRONCHIECTATIC AND NORMAL SUBJECTS

	Fever for more than 3 days	Productive Sputum	Persistent Rales	Leukocytes over 10,000	Positive Cold Agglutinins
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Bronchiectasis	70	72	75	91	7
No bronchiectasis	34	50	11	53	11

TABLE 4

CORRELATION IN POSTPNEUMONIC CASES OF BRONCHIECTASIS OF SITE OF BRONCHIECTASIS WITH SITE OF PRE-EXISTENT PNEUMONIA

Absolute correlation	20
Additional sites of pneumonia without bronchiectasis	7
Additional sites of bronchiectasis without immediately preceding pneumonia	2
Total	29

There was no significant difference between the two groups with respect to the extent of the pneumonic process as seen on the roentgenogram. A significantly greater proportion of patients in the group with bronchiectasis had prolonged fever and leukocytosis than of those in the nonbronchiectatic group, while there was no difference in the frequency of elevated cold and influenza hemagglutinin titers (table 3). Bacteriologic studies of the sputum and nasopharynx are considered to be of limited value because they were frequently obtained after antimicrobial therapy had been started.

There was a significant difference between the two groups in the physical findings in the chest. While persistent parenchymal rales for one or more weeks following the subsidence of all acute manifestations of the pneumonia were noted in 75 per cent of the patients with bronchiectasis, this was true in only 11 per cent of the patients in the nonbronchiectatic group. There appeared to be a similar, although less marked, increase in the frequency with which productive cough was present in the bronchiectatic group.

In 20 patients, there was a direct correlation between the site of the bronchiectasis and the location of the preceding pneumonia. In 7 patients bronchiectasis was found at the site of a pneumonic process, but there were additional areas of pneumonia where no bronchiectasis was noted. In 2 patients bronchiectasis was found in areas where there had been no immediately preceding roentgenographically demonstrable abnormality (table 4).

Eighteen patients had bronchograms performed for indications other than those arising during the course of an acute pneumonia. All but 3 of this group were seen initially because of acute nonpneumonic respiratory infections (pharyngitis, bronchitis), and further study was performed because of a suggestive respiratory history or abnormal chest roentgenogram. Five patients in this group were found to have bronchiectasis and thirteen had normal bronchograms.

TABLE 5

NATURE OF BRONCHIECTASIS IN THE GROUPS STUDIED

	Degree			Site			Type	
	Mild	Moderate	Severe	Right	Left	Bilateral	Cylindrical	Saccular
Recent pneumonia	12	13	4	9	10	10	27	2
Chronic history	1	2	2	1	2	2	3	2

No differences were found in the two groups with regard to past history of pulmonary disease (table 2). Rales were found in 3 of the 5 patients with bronchiectasis, but none of the normal group showed this phenomenon. Abnormal chest roentgenograms were found in 4 of 5 patients with bronchiectasis while this was true of only one of 13 in the normal group. Of interest was the fact that in the former group the roentgenographic abnormality was invariably a local one, such as localized emphysema adjacent to atelectasis.

Seven patients with bronchial asthma were bronchographed because of the presence of bothersome, chronic productive coughs. Six of these had normal bronchograms. The seventh had an irregular dilatation of an intermediate bronchus.

DISCUSSION

The results of this study indicate that bronchiectasis is a common pulmonary lesion and that its presence should be considered during the course of an acute pneumonia when certain clinical features are manifest. The most important of these features is a persistently abnormal chest roentgenogram, indicating incomplete resolution of the pneumonic process. The density should be present for at least one month and preferably for two months to be considered significant. Changes of longer duration only slightly increase the likelihood of the diagnosis of bronchiectasis.

The extent of the density has no diagnostic significance. The appearance of the abnormality is of some significance in that the presence of "peribronchial mottling" adds somewhat to the possibility that bronchiectasis is present. The presence of other roentgenographic findings, such as segmental collapse and localized emphysema or the appearance of "honeycombing," are of considerable diagnostic significance in that they suggest pre-existent pulmonary disease. Parenchymal rales that persist longer than clinical signs of activity and are out of proportion to the degree of roentgenographic abnormality are of considerable importance. Wheezes and rhonchi do not have this significance. If, in addition to the above findings, the patient's pneumonia is characterized by prolonged fever, continued productive cough, and leukocytosis, the diagnosis of bronchiectasis is rendered more likely.

The present writers agree with previous writers that although the above constellation is very suggestive, a bronchogram is warranted in every case (4). The procedure is easy to perform, carries no significant danger, and is the only objective method of demonstrating the presence and extent of the abnormality.

It would be of interest to know what happens to this type of bronchiectasis over a long period of time. Neither the present study nor other studies deal directly with this point. A different aspect of the problem has been studied by others who have shown that a variable percentage of bronchograms becomes normal if the original study is done during the course of, or soon after, an acute pneumonia.

In 1943, 5 cases of bronchiectasis that followed an acute pneumonia were shown to revert to normal within two months of the acute disease (3). In a more recent study, a group of 60 patients were bronchographed during the course of an acute pneumonia (2). Of 9 patients with "mild bronchial abnormality" in the latter series, 8 reverted to normal. Mild bronchial abnormality was described if "the bronchi showed failure of normal peripheral tapering or slight dilatation and 'beading,' but in which considerable dilatation was not present. Obstruction, incomplete filling, and absence of peribronchiolar alveolar filling, were always found associated".

In the present series only one of 14 patients with bronchiectasis, in whom the examination was repeated, showed reversion of the process to a normal state. Comparisons are difficult because of the absence of sharp criteria for the diagnosis of early bronchiectasis. This is evidenced by the fact that in 10 other patients a diagnosis was withheld until a repeat bronchogram was definitely normal. It was concluded that in the instance of the first bronchogram, the continued presence of mucus and bronchial irritation (as shown by bronchoscopy) tended to distort the bronchogram and that an original diagnosis of bronchiectasis would not have been justified. This problem was encountered even though a bronchogram was not performed until one month after all clinical evidence of activity had subsided and only after any persistent roentgenographic abnormality was shown to be stable for at least three weeks.

Undoubtedly persistent but reversible bronchial dilatation does occur after a pneumonia although the occurrence is not common in the writers' experience. Furthermore, the presence of artefacts and the difficulties inherent in borderline diagnosis should caution against overstating the problem. In any case the value of repeat bronchography has been demonstrated. Truly long term studies would be desirable to demonstrate whether or not a late reversal (more than 6 months) occurs in this group of patients. It was not possible to accomplish this in the present study.

In the group of 18 patients bronchographed because of an abnormal respiratory history, 5 patients were found to have bronchiectasis. The incidence of chronic cough and past history of pneumonia was similar in the abnormal and normal groups. Specific history of rapidly repeated bouts of pneumonia, all of which cleared rapidly, was not found to be indicative of bronchiectasis. There was no greater incidence of sinusitis in the abnormal than in the normal group. On the other hand, the roentgenographic finding of localized emphysema, segmental atelectasis, or honeycombing was confined to the bronchiectatic group. A plain film of the chest, if it shows the positive findings noted above, is therefore of some value in the pre-bronchographic diagnosis of bronchiectasis. A normal chest

roentgenogram in no way excludes the possibility of the presence of bronchiectasis.

In 1952, a report of the results of bronchography in 75 patients with bronchial asthma stated that 27 patients were found to have bronchiectasis (4). Twenty-six of the 27 patients were subjected to surgical resection, and favorable results were reported. In the present study 7 patients were selected from a much larger group with bronchial asthma because of a persistent productive cough and a history of repeated respiratory infections. Six patients had normal bronchograms and one patient demonstrated an unusual dilatation and tortuosity of an intermediate bronchus. It is believed that underlying bronchiectasis is rarely the inciting focus in patients with typical bronchial asthma.

Thus far it has been shown that bronchiectasis is found in an appreciable number of patients with pneumonia, that its presence can be suspected on clinical and roentgenographic grounds, and that the lesion is probably permanent. The most intriguing question still remains, namely, the relationship that exists between the two conditions.

Two possibilities logically explain the distinctive aspects of the pneumonia present in those patients later shown to have bronchiectasis. An underlying bronchiectasis could predispose to a more protracted course of pneumonia or, alternatively, a more severe pneumonic infection could so damage normal lung as to leave some permanent damage in the form of bronchiectasis. The only absolute method of deciding between these two explanations would involve the performance of bronchograms in unselected normal subjects and the repetition of the procedure in those who developed pneumonia. This was not done for obvious reasons.

The only other approach is an indirect one. If bronchiectasis as a disease predisposes to pulmonary infections, then the past respiratory history could be used as an index of whether bronchiectasis was present or not prior to the pneumonia. In the present study the patients whose bronchiectasis was discovered after a recent pneumonia complained of pre-existent cough or described previous pulmonary infections to no greater degree than the normal group. This may suggest that indeed the bronchiectasis was of recent origin. This line of reasoning would be strengthened if there were available for comparison a group of patients whose bronchiectasis was known to have occurred in the more distant past. Such a group was studied but unfortunately its size (5 patients) was too small to compare it with the larger group (29 patients).

A search for a "control" group in the literature yields conflicting results. In one series a large group of children who had bronchiectasis had a high percentage of pneumonic episodes and coughed more than the average child (6). On the other hand, a recent report described a series of adults with bronchiectasis whose disease was benign and not characterized by severe cough or repeated pneumonia (7). Of interest, however, is the fact that in the latter series the disease was first diagnosed in adulthood and generally after a preceding respiratory infection.

It is believed that the available evidence does not warrant a definite conclusion

on this subject. The mere association of two events does not denote a cause and effect relationship. The most that one can say is that the bronchiectasis first noted after a recent pneumonia may possibly, but not definitely, have been caused by the acute infection. Further proof will be necessary to establish this as a certainty.

Summary

Of a group of 1,711 patients with recent acute pneumonia, 69 were studied and 29 of these were found to have bronchiectasis. Bronchiectasis should be suspected when the roentgenogram shows very slow resolution of the pneumonic process, and persistence of parenchymal rales and continuation of a productive cough are observed. Bronchography is indicated regardless of the strength of clinical suspicion since it alone provides objective evidence of the presence and extent of the disease. One instance of a reversal of a previously abnormal bronchogram was discovered when repeat studies were performed on 14 bronchiectatic patients. In 10 other patients the repeat bronchograms were normal and it was concluded that the changes in the original study were due to artefacts. It is suggested that reversibility of an apparently mild bronchiectasis does occur but that this is rare, at least within several months of observation. A repeat bronchogram should, however, be done in all "borderline" cases. The relationship of the preceding pneumonia to the bronchiectasis is uncertain. While there is some evidence to suggest an etiologic role for the acute infection, this is not sufficient to be conclusive.

During the course of the study, 5 additional cases of bronchiectasis were discovered in a group of 18 patients who did not have recent pneumonia but whose clinical history suggested the disease. In 4 of the 5 positive cases there was evidence of abnormalities, such as segmental atelectasis and localized emphysema, which were present on the plain chest film.

Six of 7 patients with bronchial asthma specially selected from a larger group of asthmatic subjects because of a history of chronic cough and pulmonary infections were found to have normal bronchograms. The seventh had an unusual dilatation of an intermediate bronchus. It is concluded that bronchiectasis rarely is the inciting factor in ordinary bronchial asthma.

Sumario

Bronquiectasia y Neumonía Aguda

De un grupo de 1,711 enfermos que tenían neumonía aguda, se estudió a 69 y en 29 de éstos se descubrió bronquiectasia. Debe sospecharse bronquiectasia cuando la radiografía muestra resolución muy lenta del proceso neumónico y persistencia de estertores parenquimatosos con continuación de una tos blanda. Independientemente de la intensidad de la sospecha clínica, está indicada la broncografía, pues es la única que aporta pruebas objetivas de la presencia y extensión de la enfermedad. Se descubrió un caso de inversión de un broncograma previamente anormal al repetir los estudios en 14 enfermos bronquiectáticos. En otros 10 enfermos, los nuevos broncogramas resultaron normales, deduciéndose que las alteraciones observadas en el estudio primitivo se debían a artefactos. Sugiérese que hay reversibilidad de una bronquiectasia aparentemente leve, pero que esto es raro, a lo menos cuando sucede a los pocos meses de observación. No obstante, en todos los casos "limítro-

fes," debe repetirse la broncografía. La relación de la neumonía precedente con la bronquiectasia es incierta. Aunque existen algunos datos que sugieren un papel etiológico para la infección aguda, no alcanzan a ser terminantes.

Durante el transcurso del estudio, se descubrieron 5 casos más de bronquiectasia en un grupo de 18 enfermos que no habían tenido recientemente neumonía, pero cuya historia clínica sugería la enfermedad. En 4 de los 5 casos positivos, había signos de anomalías, tales como atelectasia segmental y enfisema localizado, que se observaban en las radiografías corrientes del tórax.

Seis de 7 enfermos de asma bronquial, escogidos especialmente de un grupo mayor de asmáticos, por tener historia de tos crónica e infecciones pulmonares, resultaron tener broncogramas normales. El séptimo tenía una dilatación inusitada de un bronquio intermedio. Dedúcese que la bronquiectasia es raramente el factor incitante en el asma bronquial ordinario.

Resume

Bronchiectasie et Pneumonie Aigue

Parmi un groupe de 1.711 malades atteints de pneumonie aiguë, 69 ont été étudiés et 29 d'entre eux ont été trouvés atteints de bronchiectasie. La bronchiectasie doit être soupçonnée quand les radiogrammes indiquent une très lente résolution du processus pneumonique, et lorsqu'on observe la persistance de râles du parenchyme et d'une toux avec expectoration. La bronchographie est indiquée, sans tenir compte de l'importance de la présomption clinique, car elle seule procure la preuve de la présence et de l'étendue de l'affection. Un cas de "conversion" d'un bronchogramme précédemment anormal a été découvert lorsque des études répétées ont été effectuées dans 14 cas de bronchiectasie. Chez 10 autres malades, les bronchogrammes répétés furent normaux et on a conclu que les modifications observées dans l'étude originale étaient dues à des artifices. L'article suggère que la réversibilité d'une bronchiectasie d'apparence légère peut survenir, mais que ceci est rare, tout au moins lorsqu'elle se produit au cours de plusieurs mois d'observations. La répétition du bronchogramme doit toutefois être effectuée dans tous les cas "limites". La relation entre la pneumonie antérieure à la bronchiectasie est incertaine. Bien qu'il existe a certains signes suggérant le rôle étiologique de l'infection aiguë, ceci n'est pas suffisant pour être concluant.

Au cours de cette étude, 5 cas additionnels de bronchiectasie ont été découverts dans un groupe de 18 patients qui n'avaient pas eu de pneumonie récente, mais dont les antécédents cliniques suggéraient l'existence de cette affection. Dans 4 des 5 cas positifs, il y avait des signes anormaux tels que l'atélectasie segmentaire et l'emphysème localisé, décelés sur les clichés ordinaires du poumon.

Parmi 7 malades atteints d'asthme bronchique ayant été choisis spécialement parmi un groupe plus grand de sujets asthmatiques, en raison d'une histoire de toux chronique et d'infection pulmonaire, on a découvert des bronchogrammes normaux chez 6 d'entre eux. Le septième présentait une dilatation anormale an niveau d'une bronche intermédiaire. Les auteurs concluent que la bronchiectasie est rarement le facteur causal dans l'asthme bronchique ordinaire.

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STUDIES ON NOCARDIA AND OTHER ACTINOMYCETALES[1, 2]

I. Cultural Studies

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Introduction

In the fields of human and veterinary medicine, the presence of Nocardia in tissues or body products may sometimes create confusion in diagnosis and prognosis unless the true nature of the organisms is recognized early. While some organisms are merely contaminants or secondary invaders as are certain saprophytic Mycobacteria, other Nocardia are undoubtedly capable of causing serious or even fatal disease so that their immediate accurate identification may be important. Current taxonomic classifications of Nocardia include enumeration of many species, based in part at least on the earlier literature. Unfortunately, in some of the older articles the descriptions are grossly inadequate by modern standards or are based on only a few species of which the original type of strains are no longer available for comparative purposes. The deficiencies of existing keys become immediately apparent when one attempts to use them. In many instances categories leading to specific identification are based on differences in the degree of a single morphologic, tinctorial, or cultural property while numerous investigations (1–10) have served to emphasize the often marked extent to which such properties may vary.

The present study was undertaken in the hope that it would shed additional light on the value of various characteristics as criteria for specific identification, with the ultimate goal of simplifying the taxonomy of the Nocardia. With this goal in mind, a collection of strains previously identified as Nocardia, including both saprophytic and known pathogenic strains, were obtained from various sources. For comparative study certain Mycobacteria were studied as well as a few species of Streptomyces. The last-named species were included because the existing knowledge seemed inadequate with regard to the relationships among these genera. The collection was examined for behavior, employing methods which it was believed might yield data of differential value. This was done particularly since, for purposes of uniformity, the various strains were inoculated into samples of a given batch of medium at approximately the same time. The present paper is intended primarily to summarize the cultural findings; later communications will present the results of serologic studies.

Materials and Methods

The organisms used, together with their original strain designations and sources,[3] were as follows:

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[1] From the Department of Microbiology, Tulane University School of Medicine, New Orleans, Louisiana.

[2] This work was supported by a contract between the Office of Naval Research, Department of the Navy, and Tulane University.

[3] The sources indicated by the letters in parentheses were: (*a*) USPHS Hospital, New

Nocardia asteroides: strains—Wojik 23 (*a*); Henrici 4139 (*b*); Henrici A10A (*c*); ATCC 3308, ATCC 9504, ATCC 9970 (*d*); CDC 47, CDC 144, CDC 267, CDC A343, CDC A402 (*e*); 14A1 (*f*); 47295 (*g*); 2001 (*h*); 356 (*i*); ATCC 9969 (*j*); 359 (*k*); NRRL B 970 (*l*); *N. blackwellii* ATCC 6846 (*j*); *N. brasiliensis* (*N. mexicana*): strains—Ochoa 409, Ochoa 414, 2178 (*b*); 413, 992 (*e*); 47296 (*g*); *N. caprae* 373 (*l*); *N. caviae* ATCC 6848 (*d*); *N. convoluta* ATCC 4275 (*l*); *N. corallina* ATCC 999 (*m*); *N. cuniculi* ATCC 6864 (*j*); *N. erythropolis* ATCC 4277 (*j*); *N. gardneri* ATCC 9604 (*j*); *N. globerula* ATCC 9356 (*d*); *N. intracellularis* 330 (*b*); *N. leishmanii* ATCC 6855 (*j*); *N. madurae*: strains—Ochoa 415 (*b*); ATCC 6245 (*n*); CDC 434 (*e*); Aldana 339 (*a*); *N. minima* ATCC 8674 (*d*); *N. opaca* ATCC 4276 (*d*); *N. paraffinae* 3410 (*m*); *N. pelletieri*: strains—Lacaz 293 (*o*); 47293 (*q*); *N. polychromogenes* ATCC 3409 (*j*); *N. rangoonensis* ATCC 6860 (*j*); *N. rubra:* strains—NRRL B685, 379 (*l*); *N. sylvodorifera* ATCC 7372 (*l*); *Mycobacterium butyricum* 264 (*p*); *M. fortuitum* ATCC 6841 (*q*); *M. moelleri* 268 (*p*); *M. leprae*: strains—Duval 104, Duval 129 (*p*); *M. phlei*: strains—W23 (*q*), 125 (*p*); *M. smegmatis*: strains—270 (*p*); NCTC 8150 (*q*); *M. stercoris* 262 (*p*); *Streptomyces aureus* ATCC 3309 (*j*); *S. griseus* ATCC 3326A (*j*); *S. ruber* ATCC 3348 (*j*); *S. venezuelae* ATCC 10595 (*j*).

Stock cultures were maintained on beef infusion 4 per cent glycerol agar slants pH 6.8, transplanting every six to eight weeks. Other solid media used in the examination of gross and microscopic morphology were: Sabouraud's glucose agar (11), carrot agar (12), Czapek's agar (13), potato glucose agar (14), and starch agar (neopeptone, 10 gm.; glucose, 5 gm.; NaCl, 1 gm.; Difco soluble starch, 10 gm.; agar, 20 gm.; distilled water, 1,000 ml.). All cultures were incubated at 25°C. except in the tests conducted for their ability to grow at 46° C. or higher temperatures.

In testing for acid-fastness, growth from a two-week-old culture in skim milk (Difco) or other medium was spread thinly on a slide and stained at room temperature by Kinyoun's method. Decolorization was attempted by flooding the slide with 2 per cent HCl in 95 per cent ethanol for three to five seconds; smears were then washed with water and counterstained with methylene blue. Only very thin portions of the smear were examined for acid-fast elements.

To detect paraffin utilization a small particle of growth from glycerol agar was emulsified in a tube containing Czapek's fluid without added carbohydrate. A glass rod, coated with sterile paraffin, was then inserted in the fluid so that at least one centimeter remained above the surface. The cultures were observed for evidence of growth on the paraffin at intervals up to four weeks.

For determination of diastatic activity, sets of cultures streaked down the center of the surface of long thin slants of starch agar were tested after both ten and twenty-five days by applying 1 to 2 ml. of iodine solution and reading after a five-minute interval.

At intervals of fourteen, thirty, and forty days, respectively, after inoculation, sets of nutrient gelatin cultures in Wassermann tubes were observed for persistent liquefaction.

For detecting casein hydrolysis, Hastings' milk agar plates were used according to the method of Gordon and Smith (15).

Tubes of urea broth (Difco) were inoculated with a large loopful of culture emulsion, held at 25° or 37° C., and examined periodically for three weeks to detect urease activity.

Catalase activity and indol production were determined by tests on two-week-old cultures on glycerol agar and in N-Z broth (Sheffield), respectively.

Orleans; (*b*) Dr. N. F. Conant, Duke University; (*c*) Department of Bacteriology, University of Minnesota; (*d*) U. S. Army Medical Center; (*e*) USPHS Communicable Disease Center, Atlanta; (*f*) Dr. A. Kligman, University of Pennsylvania; (*g*) N. Y. State Department of Health, Albany; (*h*) Dr. S. Salvin, USPHS Rocky Mountain Laboratory; (*i*) Department of Veterinary Pathology, Ohio State University; (*j*) American Type Culture Collection; (*k*) Dr. J. Carter, Kansas City General Hospital; (*l*) Dr. N. M. McClung, University of Kansas; (*m*) Dr. R. L. Starkey, Rutgers University; (*n*) Dr. E. H. Ludwig, University of West Virginia; (*o*) Dr. A. Gonzalez Ochoa, Mexico City, D.F.; (*p*) Department of Microbiology, Tulane University; (*q*) Dr. R. Gordon, Rutgers University.

Two sets of cultures were prepared on nitrate agar and tested for production of nitrites after ten or thirty days.

Plates of 3 per cent sheep blood heart infusion agar were examined over a three-week period for evidence of hemolysis.

Results

All strains were studied for the following characteristics: (*a*) pigmentation of the surface growth (stroma); (*b*) diffusible pigment production; (*c*) production of aerial mycelium; (*d*) gross appearance and consistency of the stroma on solid media; (*e*) microscopic characteristics in slide culture; (*f*) acid-fastness; (*g*) growth at 46° C.; (*h*) paraffin utilization; (*i*) diastatic activity; (*j*) gelatin liquefaction; (*k*) casein hydrolysis; (*l*) urease activity; (*m*) catalase activity; (*n*) indol production; and (*o*) hemolytic activity. In addition, 25 selected strains of Nocardia and 5 strains of Mycobacterium were examined for nitrate reduction and also for ability to produce aerial mycelium on Czapek's agar, Sabouraud's agar, blood agar, potato glucose agar, carrot agar, and Pai's coagulated egg medium, as well as on the glycerol and starch media on which aerial mycelium production was observed for all strains. Most of the results are summarized in tables 1 and 2. Despite presentation of the data in the respective tables, certain further explanatory and analytical comments seem warranted.

As expected, pigment production of the stroma varied to some extent with the composition of the growth medium (10), age of culture, and temperature of incubation, being generally paler in the cultures at 46° C. than in those at 25° C. Coloration remained relatively constant for the first four to five weeks of growth, then began to darken. For most strains of *N. asteroides*, pigments ranged from yellow to red-orange, one exceptional strain produced a tan pigment. Many other species of Nocardia as well as most of the Mycobacterium strains produced similar yellow to orange, as well as red, pigments. The species of Streptomyces studied, as well as some species of Nocardia, usually produced cream, tan, or brown (only rarely yellow, orange, or red) pigments.

The ability to produce a diffusible pigment in the medium varied greatly among the cultures studied and did not appear to be correlated with any other cultural characteristic. Certain strains of Nocardia and Streptomyces produced a diffusible pigment on all of the various media examined, some strains on none and others on one or more of the media. The Mycobacterium species did not produce such pigment in any of the media. In addition, pigment production sometimes varied for a single strain on the same medium. A few strains, e.g., *N. paraffinae*, lost their ability to produce a diffusible pigment on glycerol agar during three years of subculture (2). Other strains, e.g., *N. madurae* ATCC 6245, *N. brasiliensis* Ochoa 409, *N. polychromogenes*, and *N. rangoonensis*, apparently acquired this ability during the same interval, when tested on starch agar.

Aerial mycelium visible to the unaided eye was produced by many strains of Nocardia but was quite variable for each strain and depended to a large extent on the medium used as well as on the length of time the organisms had been maintained in subculture. It was found (tables 1, 2) that Nocardia strains were

more likely to produce an aerial mycelium on starch agar slants than on any other of the media tested. Most of the strains listed in table 2 that produced an aerial mycelium on glycerol agar in 1952 failed to do so in 1955, and some strains even lost their ability to produce aerial mycelium on starch agar after three years of subculture.

In slide cultures, using potato glucose agar, all Nocardia strains except *N. intracellularis* produced a well-developed mycelium within the substrate (submerged mycelium). In the initial stages of growth they also produced above the surface of the medium a branched mycelium, which in many cases fragmented into coccoid or bacillary elements. These commenced at the agar surface and proceeded toward the tips of the hyphae as the culture aged. In some strains, e.g., *N. globerula* and *N. corallina*, fragmentation proceeded so rapidly that after three to four days of growth no evidence of a true branched mycelium remained. Other strains showed fragmentation as well as abundant branched mycelial elements regardless of the age of the culture. *N. intracellularis* produced an almost completely bacterial type of growth; in slide culture a branched mycelium was not produced either within or above the surface of the substrate. A few strains of Nocardia and four species of Streptomyces did not fragment in slide culture; these strains were invariably nonacid-fast.

The Mycobacterium strains in slide culture showed only a packed mass of bacillary elements on the surface and no submerged growth, with the exception of *M. smegmatis* 270 and *M. leprae* Duval 129, both of which showed branched mycelial elements in the surface growth. *M. leprae* Duval 129 also produced a submerged mycelial growth, was not acid-fast, and appeared indistinguishable from *N. rubra* 379.

The tendency or failure of the mycelium to fragment was closely correlated with the gross appearance of the growth on solid media. The strains that produced true fragmentation of the mycelium also produced either a soft bacterium-like growth or a firmer, waxy, verrucose, friable crumbly growth; the latter emulsified with difficulty and usually showed an aerial mycelium on starch agar. A few strains showed an intermediate gross morphology. The nonfragmenting, nonacid-fast strains of Nocardia and Streptomyces appeared quite different grossly from the fragmenting strains of Nocardia. Both nonfragmenting varieties produced on solid media either a thin, often translucent, flat, wrinkled membrane-like stroma or a thicker, opaque, usually leathery stroma. In a few of the nonfragmenting strains the stroma was soft. Two strains of *N. madurae* (Ochoa 415 and Aldana 339) and both strains of *N. pelletieri* had a gross morphology somewhat intermediate between that of the streptomyces-like strains of Nocardia and the fragmenting Nocardia.

The degree of acid-fastness exhibited by Nocardia strains appeared to depend to some extent on the age of the culture, on the propagating medium, and on the staining procedure employed. In the preliminary stages of the investigation the observation of others (5, 16, 17) was confirmed that the largest number of strains of Nocardia were acid-fast when grown in fluid milk. Among solid media, glycerol agar yielded the highest percentage of strains exhibiting acid-fastness as com-

TABLE 1

CULTURAL PROPERTIES OF STRAINS OF NOCARDIA, STREPTOMYCES, AND MYCOBACTERIUM

Strain	Gross Culture				Slide Culture*			Physiologic and Biochemical Properties							
	Color of Stroma				Surface Growth										
	Yellow, Orange, Red	White, Cream, Tan, Buff, or Brown	Diffusible Pigment*	Aerial Mycelium*	Branched Mycelium	Fragmentation	Submerged Growth Present	Acid-fastness	Growth at 46°C	Paraffin Utilization	Diastasis	Gelatin Liquefaction	Casein Hydrolysis	Urease	Hemolysis
Nocardia															
N. asteroides Wojik 23	+	−	+	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides Henrici 4139	+	−	+	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides Henrici A10A	+	−	+	+	+	+	+	+	+	+	−	−	−	−	+
N. asteroides ATCC 9504	+	−	+	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides ATCC 9970	+	−	−	−	+	+	+	+	+	+	−	−	−	+	−
N. asteroides ATCC 3308	+	−	−	+	+	+	+	−	+	+	−	−	−	+	+
N. asteroides CDC A343	+	−	+	−	+	+	+	+	+	+	−	−	−	+	+
N. asteroides 14A1	+	−	+	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides 47295	+	−	+	−	+	+	+	+	+	+	−	−	−	+	−
N. asteroides CDC A402	+	−	−	+	+	+	+	+	+	+	−	−	−	±	−
N. asteroides CDC 47	+	−	+	+	+	+	+	−	+	+	−	−	−	+	−
N. asteroides CDC A144	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−
N. asteroides CDC A267	+	−	−	+	+	+	+	+	+	+	−	−	−	−	−
N. asteroides 2001	+	−	−	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides 356	+	−	−	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides ATCC 9969	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−
N. asteroides 359	+	−	−	+	+	+	+	+	+	+	−	−	−	−	−
N. asteroides NRRL B970	+	−	+	+	+	+	+	+	+	+	−	−	−	+	+
N. blackwellii ATCC 6846	+	−	−	−	+	+	+	+	+	+	−	−	−	+	−
N. caprae 373	+	−	+	−	+	±	+	+	+	−	−	−	−	+	−

| | | |

TABLE 1—Continued

Strain	Gross Culture				Slide Culture*			Physiologic and Biochemical Properties							
	Color of Stroma		Diffusible Pigment*	Aerial Mycelium*	Surface Growth		Submerged Growth Present	Acid-fastness	Growth at 45°C	Paraffin Utilization	Diastasis	Gelatin Liquefaction	Casein Hydrolysis	Urease	Hemolysis
	Yellow, Orange, Red	White, Cream, Tan, Buff, or Brown			Branched Mycelium	Fragmentation									
Mycobacterium															
M. butyricum 264	−	+	−	−	−	+	−	+	+	+	−	−	−	+	−
M. fortuitum ATCC 6841	−	+	−	−	−	+	−	+	+	+	−	−	−	+	−
M. moelleri 268	−	+	−	−	−	+	−	+	+	+	−	−	−	+	−
M. leprae Duval 104	+	−	−	−	−	+	−	+	+	+	−	−	−	+	−
M. leprae Duval 109	+	−	−	−	+	+	+	−	+	+	−	−	−	+	−
M. phlei W23	+	−	−	−	−	+	−	+	+	+	−	−	−	+	−
M. phlei 125	+	−	−	−	−	+	−	+	+	+	−	−	−	+	−
M. smegmatis 270	+	−	−	−	±	+	−	+	+	+	−	−	−	+	−
M. smegmatis NCTC 8150	+	−	−	−	−	+	−	+	+	+	−	−	−	+	−
M. stercoris 262	−	+	−	−	−	+	−	+	+	+	−	−	−	+	−

* Tested on starch agar.

pared with blood agar, starch agar, Sabouraud's agar, and potato glucose agar. When decolorized with 1 per cent aqueous H_2SO_4, more strains appeared acid-fast, but there was difficulty in evaluating the smears since nonacid-fast forms appeared reddish-purple instead of blue and it was believed that the results obtained using H_2SO_4 were too variable. The results presented in table 1 were obtained using HCl as decolorizer and flooding the slide with acidified alcohol for three to five seconds or until the red dye ceased to dissolve from the thin portion of the smear. Of the 18 strains of *N. asteroides* examined, two which were otherwise typical (ATCC 3308 and CDC 47) could not be shown to be acid-fast despite repeated attempts. As Waksman and Le Chevalier (18) have noted,

TABLE 2

EFFECT OF MEDIUM ON AERIAL MYCELIUM FORMATION BY SELECTED STRAINS OF NOCARDIA AND MYCOBACTERIUM

Strain	Aerial Mycelium on:					
	Carrot Agar	Czapek's Agar	Pai's Medium	Potato Glucose Agar	Sabouraud's Agar	Glycerol Agar
Nocardia						
N. asteroides Wojik 23	+	+	−	+	+	+
N. asteroides Henrici 4139	−	+	+	+	+	−
N. asteroides Henrici A10A	+	+	+	−	+	+
N. asteroides ATCC 9504		+	+	+	+	+
N. asteroides ATCC 9970	+	+	+	+	+	+
N. asteroides ATCC 3308		−	+	−	+	+
N. blackwellii ATCC 6846	−	−	−	−	+	−
N. cuniculi ATCC 6864	−	−	−	−	−	+
N. minima ATCC 8674	+	+	+	+	+	+
N. paraffinae 3410	−	+	−	−	−	−
N. polychromogenes ATCC 3409	+	+	+	+	+	+
N. brasiliensis Ochoa 409	+	+	−	+	−	+
N. brasiliensis 2178	+	+	+	+	+	+
N. brasiliensis Ochoa 414	+	+	+	+	+	+
N. corallina ATCC 999	−	−	−	−	−	−
N. erythropolis ATCC 4277	−	−	−	−	−	−
N. globerula ATCC 9356	−	−	−	−	−	−
N. opaca ATCC 4276	−	−	−	−	−	−
N. caviae ATCC 6848	−	−	+	−	+	+
N. leishmanii ATCC 6855	+	−	−	+	−	−
N. madurae Ochoa 415	+	−	−	+	−	−
N. madurae ATCC 6245	+	+	+	+	+	+
N. pelletieri Lacaz 293	−	−	−	−	−	−
N. rangoonensis ATCC 6860	+	+	+	+	+	+
N. intracellularis 330	−	−	−	−	−	−
Mycobacterium						
M. butyricum 264	−	−	−	−	−	−
M. leprae Duval 104	−	−	−	−	−	−
M. phlei 125	−	−	−	−	−	−
M. smegmatis 270	−	−	−	−	−	−
M. stercoris 262	−	−	−	−	−	−

N. leishmanii also appeared nonacid-fast although according to the original description it was a markedly acid-fast isolate. The single strains of *N. erythropolis* and *N. corallina* which were tested, as well as 1 of 2 *N. rubra* strains, showed acid-fast properties although these species are generally considered to be nonacid-fast.

While the majority of actinomycetes are considered to be mesophilic, with 40° C. the maximal temperature for growth (19), many Nocardia strains in the present series, including isolates from human and animal sources, developed profusely at 46° C. while the *N. brasiliensis* strains and certain saprophytic Nocardia uniformly failed to do so. Even at 50° C. the *N. blackwellii*, *N. caprae*, *N. cuniculi*, *N. sylvodorifera* strains and 11 of the 22 strains of *N. asteroides* tested were found to show definite growth within four days of incubation (17). This characteristic appeared to be stable in tests repeated after an interval of three years. The saprophytic mycobacteria examined grew at 46° C., as expected (20–22); they also developed at 50° C., with the exception of *M. fortuitum* and the 2 strains labelled *M. leprae*.

Most strains of Nocardia appeared capable of utilizing paraffin, also some of the streptomyces-like organisms; *N. caprae* and certain of the streptomyces-like Nocardia were exceptional in not utilizing paraffin. These positive results were largely similar to those of McClung's extensive study (8) except for *N. leishmanii* ATC 6855; negative findings have been reported for a minority of *N. asteroides* and *N. brasiliensis* strains (23). Paraffin served to support growth of all of the limited series of Mycobacterium strains examined (20, 24) including *M. phlei* and *M. butyricum* (syn. *M. lacticola*), although Penso (21) regards these two species as incapable of metabolizing the material as a carbon source. The ability to utilize paraffin thus appeared to be of only limited differential value among Nocardia and Mycobacteria.

All organisms tested failed to produce indol (22).

It was of interest that all of the strains of Nocardia, Streptomyces, and the Mycobacteria examined uniformly showed catalase activity. This property of the latter group is well known (22, 25) but has recently been demonstrated to be altered among isoniazid-resistant strains of tubercle bacilli (26) and absent among strains of *Actinomyces bovis* (27).

Since it was anticipated that some of the strains to be examined for diastatic activity might be unable to develop on a substrate in which starch was the sole carbohydrate source, a medium was employed that contained in addition an amount of glucose which served to promote good growth in each instance. Even under these circumstances, the Mycobacterium species uniformly failed to show starch hydrolysis (24, 28) as did most of the Nocardia, the exceptions being *N. caviae*, *N. gardneri*, *N. leishmanii*, and 3 of the 4 *N. madurae* strains (8, 24, 28–30). Amylolysis was regularly demonstrated with the few Streptomyces species tested, in conformity with the work of previous investigators (13, 19 31). It may be mentioned that McClung (7) found a single strain of *N. caviae* and 2 of 6 *N. asteroides* strains unable to utilize starch, while Mariat and Lavalle (23) recorded 1 of 11 *N. asteroides* strains and 5 of 10 *N. brasiliensis* strains as

utilizing this carbohydrate for growth; these investigators did not, however, test directly for diastatic activity.

The strains examined for gelatin liquefaction and casein hydrolysis yielded either negative or positive results for both activities. The 4 species of Streptomyces, all of the streptomyces-like Nocardia and all strains of *N. brasiliensis* showed proteolytic activity in contrast to the remaining Nocardia strains and the strains of Mycobacterium; these findings were in agreement with the earlier pertinent literature (15, 16, 29, 30, 32).

Most of the acid-fast Nocardia strains and all species of Mycobacterium tested were urease positive, whereas the majority of the nonacid-fast streptomyces-like Nocardia and 3 of the 4 species of Streptomyces were urease negative. Two Nocardia strains showed considerable variability of urease activity (indicated in table 1 by ±) when tests were run in duplicate and repeated numerous times. Three strains (*N. corallina*, *N. erythropolis*, and *N. globerula*) were urease positive within six hours' incubation, while 5 strains of *N. asteroides* were urease positive at eighteen hours; additional strains became positive at varying intervals of time up to but not after the sixth day of incubation. In table 1 the results recorded are those observed after six days.

While the 4 species of Streptomyces and strains of the streptomyces-like Nocardia were for the most part actively hemolytic on sheep blood agar, only 3 strains of the acid-fast fragmenting Nocardia produced hemolysis within five days of incubation; however, additional strains showed hemolysis as the cultures aged. The ability to produce hemolysis did not appear to be related to any other physiologic activity. No hemolytic activity was observed in any of the Mycobacterium strains tested.

Although the intensity of nitrate reduction showed considerable variability among strains, in the limited series examined the ability to reduce nitrate to nitrite did not appear to be characteristic of any particular group of strains that otherwise seemed to be related on the basis of cultural similarities.

Discussion

Although the growth produced by all actinomycetes above the surface of solid media is literally an "aerial mycelium," the term is usually used in a more restricted sense. The filaments produced during initial stages of growth of Nocardia and Streptomyces species eventually come to lie chiefly parallel to the surface of the medium and branch profusely, forming a fairly compact stroma. In some strains short, vertically erect filaments which constitute the aerial mycelium proper arise from the stroma, giving the surface a fuzzy, chalky, or powdery aspect. The stroma is sometimes referred to as the vegetative mycelium, particularly among the streptomycetes in which the aerial mycelium is a sporulating mycelium; in the absence of sporulation, however, both stroma and aerial mycelium could be called vegetative (33). To avoid confusion in terminology, the term "stroma" is used in this paper in preference to "vegetative mycelium."

"Fragmented mycelium" as used here refers to an active segmentation of hyphae into bacillary or coccoid elements. When fragmentation involves most of the mycelium, a soft pasty type of growth is produced, but it has been found that in some strains such growth is not associated with the production of bacillary and coccoid forms. Certain

strains of the streptomyces-like Nocardia, e.g., *N. gardneri*, produce very fragile hyphae that break apart easily when manipulated with an inoculating wire; smears made from such strains show numerous branched mycelial fragments but not bacillary or coccoid elements. In slide culture, where the mycelium can be observed intact, spontaneous fragmentation does not occur. In addition, autolytic liquefaction of the older basal portion of the stroma or possibly the secretion of gelatinous material may also contribute to the formation of a soft growth which is not restricted to any one morphologic group. Slide culture is usually advisable (and often necessary) to determine whether or not such strains are producing true mycelial fragmentation. The soft, fragmenting, acid-fast Nocardia are often grossly indistinguishable from Mycobacteria, differing in colonial morphology from the latter only in their ability to produce a submerged mycelium in slide culture.

No marked correlation was observed between other cultural characteristics and aerial mycelium production; among the acid-fast Nocardia strains, those which produced almost complete mycelial fragmentation early in growth did not produce an aerial mycelium on any type of medium. However, some of the nonfragmenting, nonacid-fast Nocardia also failed to produce an aerial mycelium.

Although in the present experiments a neopeptone glucose agar containing starch appeared to favor aerial mycelium formation by many of the strains, no single medium of the several varieties tested regularly elicited such behavior by all of the strains which were found to be capable of producing aerial hyphae. Previous workers have also noted the advisability of employing several different media in order to demonstrate aerial mycelium among the largest proportion of a collection of streptomycetes. Erikson (4, 5, 34) has analyzed some of the factors which may affect the production of aerial mycelium by a given strain. Because the production of aerial mycelium is an unstable characteristic among Nocardia, it is of only limited value in species determination. However, inasmuch as the Mycobacteria have only very rarely been observed to produce them on any medium, one might be justified in tentatively eliminating from consideration as a Mycobacterium any acid-fast aerobic organism showing an evident aerial mycelium or submerged mycelium.

In contrast to the above-mentioned bacillary and coccoid forms (oidia) derived by fragmentation in the Nocardia, the characteristic spores of streptomycete species have been defined as "bodies identical to one another in form and having a special mode of formation" (35). Such spores (conidia) are produced in chains on special branches of the aerial mycelium, arising from the tips of growing hyphae, the basal portion of which remains unfragmented (13, 36, 37). Conidia production among the streptomycetes is a fluctuating characteristic, however, and some strains lose their ability to produce spores on subculture (3, 38, 39). No conidia were observed in slide cultures of any of the strains studied here, including the 4 species of Streptomyces that were presumably identified originally on the basis of spore production. This suggests that some of the nonfragmenting, nonacid-fast strains designated as Nocardia might actually be species of Streptomyces which had lost their ability to produce spores but remained otherwise culturally unchanged.

It should be noted that a large collection of strains, received from various sources and designated as Nocardiae, has recently been examined by Gordon and Mihm (17) who considered that 79 of the cultures satisfied their criteria for classification as *N. asteroides*. Among this latter group, 21 "segmented into chains of even, beadlike spores." Since *N. asteroides* has been generally regarded as failing to produce conidia, it is regrettable that these workers did not illustrate their observation or indicate whether the spores they observed were regarded as oidia or conidia, or specify the individual strains which appeared to sporulate. While the proposal by Gordon and Smith (15) to separate Nocardia from Streptomyces on the basis of biochemical activities rather than spore formation remains to be evaluated further, it would seem logical to reclassify as Streptomyces those strains previously designated as Nocardia but which exhibit most of the physiologic behavior common to the genus Streptomyces and additionally possess an identical gross and microscopic morphology.

As with other microbes, color and intensity of stromal pigment produced by the strains in this collection have proved to be variable characteristics. Moreover, while it is possible to separate the strains into two color groups, i.e., yellow-orange-pink-red and cream-tan-brown, these overlap somewhat. The elaboration of diffusible brownish to black pigment is likewise unpredictable (40) although it is more frequently shown during growth on media rich in tyrosine (41), and thus does not permit the definitive grouping of strains of Nocardia or Streptomyces. Such inconstancy may account for the fact that, although the species is described (18) as failing to do so, many of the *N. asteroides* strains in the collection of the present writers did produce soluble pigment (17). Gordon and Mihm (17) and Haag (20) have recorded certain strains which they considered to be Mycobacteria capable of producing a diffusible melanin; but none of those included in the present study ever exhibited this property, the association of such pigment with an isolate would seem rather to plead against its belonging to the genus Mycobacterium.

The crucial importance of the particular technical method employed for biochemical assay was re-emphasized in the tests for diastatic and proteolytic activity. Thus, the data for amylolysis, utilizing Difco soluble starch as substrate and iodine as test reagent, were in general accord with most of the earlier literature but differed significantly from the findings of Gordon and co-workers (17, 42) who reported *M. fortuitum*, *M. phlei*, and *M. smegmatis* strains to be regularly diastatic as well as roughly half of the strains they considered to be *N. asteroides*; their method, however, involved the use of potato starch and alcohol as reagent. In the tests in the present studies there was complete correlation between gelatin liquefaction and casein hydrolysis. For both reactions, negative results were uniformly obtained with the Mycobacteria and also Nocardiae except for *N. brasiliensis* and certain streptomyces-like Nocardia. By contrast, Gordon and co-workers, using a different test method, found roughly a quarter of their *M. phlei* and a third of their *N. asteroides* strains to hydrolyze gelatin, although none decomposed casein.

In regard to urease activity, the present findings have confirmed and extended those of earlier workers (13, 19, 23, 30, 43–45) who noted that: (*a*) the majority

of strains of *N. asteroides*, *N. brasiliensis*, and *N. madurae* give positive reactions; (*b*) other Nocardia and Streptomyces species often yield negative results; (*c*) *M. tuberculosis* (especially the avian type) elaborates the enzyme but the property has not been extensively investigated among other mycobacterial species. In the present comparative study the high degree of activity shown by *N. corallina* and certain related species was striking (46). Additional work is in progress to explore the application of the urease test in identification of Nocardia, as Seeliger (47) has done for cryptococci.

On the basis of the cultural data presented in the tables and the fore-going discussion, the present writers venture to tentatively classify the strains of Nocardia examined as follows:

A. "True" Nocardia

Aerobic actinomycetes whose mycelium actively fragments into bacillary and coccoid elements, beginning in the basal portion of the hyphae and proceeding toward the tip. Aerial mycelium often produced, usually white but occasionally pink. A branched mycelium is produced within the substrate. Stroma was some shade of yellowish-tan, yellow, orange, pink, or red. Some degree of acid-fastness manifested by many strains but may be absent; nondiastatic. All strains except *N. brasiliensis* fail to liquefy gelatin or hydrolyze casein; occasionally hemolytic on blood agar; divided into the following groups:

I. *N. asteroides* group: Stroma elevated, relatively firm, wrinkled to verrucose, cartilaginous, waxy, crumbly, or friable, only rarely soft. Not readily emulsified in saline; good growth at 46° C.; most strains urease positive within six days. Includes all strains of *N. asteroides* studied as well as *N. blackwellii* ATCC 6846, *N. cuniculi* ATCC 6864, *N. caprae* 373 and *N. sylvodorifera* ATCC 7372. *N. polychromogenes* ATCC 3409, *N. minima* ATCC 8674, and *N. paraffinae* 3410 are culturally identical with *N. asteroides* strains except for inability to grow at temperatures above 40° C. and there is doubt as to the advisability of separating them solely on the basis of this property.

II. *N. brasiliensis* group: Similar in gross appearance to *N. asteroides*. Growth very firm, finely wrinkled to almost filiform on the surface, strongly adherent to the substrate. Branched mycelium in slide culture extensive with fragmentation confined to the extreme basal portion; not readily emulsifiable in saline. No growth at 46° C.; gelatin liquefied and casein hydrolyzed; urease positive. Includes only the six strains of *N. brasiliensis* studied.

III. *N. corallina* group: Stroma on solid media always soft and pasty, surface flat with few wrinkles but finely papillose, resembling the surface of a tongue. Stromal pigment pale peach or pale orange. In slide culture, fragmentation of the mycelium is extensive and eventually branched mycelial elements are recognizable only at the outermost fringes of the surface growth. Aerial mycelium never produced on any medium; easily emulsified in saline. No growth at 46° C.; urease positive. Includes *N. convoluta* ATCC 4275, *N. corallina* ATCC 999, *N. erythropolis* ATCC 4277, and *N. globerula* ATCC 9356. All 4 strains are culturally identical and are probably variants of a single species.

IV. *N. opaca* group: Stroma soft and pasty; orange-red to deep red, surface flat, smooth, and shiny, but often with numerous "worm-like" wrinkles. Slide-culture characteristics identical with those of the *N. corallina* group; no aerial mycelium on any medium. Good growth at 46° C.; urease negative. Includes *N. opaca* ATCC 4276 and both strains of *N. rubra*.

B. Streptomyces-like Nocardia:

Aerobic actinomycetes producing an extensive branched mycelium that does not actively fragment into bacillary or coccoid forms although with age it may sometimes break up into shorter branched elements. Stroma pale cream or ivory, tan to deep brown, occasionally pink, red, or yellowish; gross appearance of the stroma quite variable; occasionally soft but usually producing a tough, flat, sheetlike leathery growth or a thin, translucent, membranous growth; sometimes elevated, cartilaginous, or cerebriform; surface usually wrinkled with an arachnoid or honeycomb appearance. Aerial mycelium usually produced on starch agar. Never acid-fast on any medium; most strains diastatic. All strains liquefy gelatin and hydrolyze casein; most strains urease negative; often hemolytic. A very heterogeneous group, with cultural and morphologic characteristics similar to those of many species of *Streptomyces*. Because of the small number of strains studied no practical division of this group on a cultural basis is attempted.

Among the streptomyces-like strains studied *N. madurae* (Ochoa 415 and Aldana 339) and *N. pelletieri* (Lacaz 293 and 47293) have almost identical cultural characteristics, except for the production by *N. pelletieri* of a deep ruby-red pigment on starch agar or Pai's egg medium as compared to the ivory pigment produced by *N. madurae* on the same media. The clones used of *Streptomyces venezuelae* ATCC 10595, *N. gardneri* ATCC 9604, and *N. madurae* ATCC 6245 and CDC 434 are identical in the gross appearance of the stroma, which is flat with arachnoid wrinkling and deep brown; these two strains of *N. madurae* do not correspond to the type strain described in *Bergey's Manual* (48).

N. caviae ATCC 6848, *N. rangoonensis* ATCC 6860, and *N. leishmanii* ATCC 6855 are quite similar in appearance to the clone of *S. griseus* ATCC 3326A used in this laboratory, but *N. rangoonensis* differs in its inability to hydrolyze starch and *N. leishmanii* differs in its inability to grow at 42° C. The latter strain is brown, does not fragment, is not acid fast, and is actively proteolytic so that obviously this strain is not the same *N. leishmanii* as was described in the older literature (48, 49). Similarly, the present observations on the properties of *N. caviae* ATCC 6848 do not agree with a previous description of this species (48), in which fragmentation, the production of a pink stroma, and the occasional formation of chains of conidia are mentioned. For the present, the true identity of the questionable strains discussed above remains undetermined.

In the recent study of Gordon and Mihm many of the same strains were examined as in the present investigation, although some were obtained from other laboratories and had undoubtedly been maintained under varying conditions. Their taxonomic conclusions differed from those of the present study in the following particulars:

(*a*) *N. corallina* W3409 (ATCC 999 of the present series), *N. erythropolis* ATCC 4277, *N. globerula* ATCC 9356, *N. opaca* ATCC 4276, *N. rubra* strains 415 (NRRL B-685 of the present series) and 562 (379 of the present series) were re-classified as *Mycobacterium rhodochrous*; however, since these strains were found to produce submerged mycelium in contrast to the mycobacteria tested, the former are regarded as "true" Nocardia. It may be pointed out also that on cytochemical analysis, *N. rubra* 3639 which Gordon and Mihm classified as *M. rhodochrous*, was found to have a cell-wall carbohydrate com-

position very similar to that of *N. polychromogenes* 3409 and *N. asteroides* 3573; the latter, incidentally, was observed to produce evident aerial mycelium with conidia (50).

(*b*) *N. gardneri* N6531 (ATCC 9604 of the present series) was considered to be a strain of *N. asteroides*, whereas the behavior of the clone in the present series seems rather to justify inclusion with the streptomyces-like Nocardia.

The status of *N. intracellularis* deserves brief consideration. As isolated and first described (51), the organism grew on Sabouraud's agar at room temperature and in slide culture produced evident branching but without aerial hyphae or conidia. The culture received several years later, although still exhibiting many of the original properties, has uniformly failed to produce branching or submerged mycelium and it now behaves much more like a Mycobacterium. Furthermore, in serologic studies this strain has been found to be quite unrelated to any of the "true" Nocardia with which it has been compared, exhibiting in fact a high degree of antigenic individuality.

Summary

Comparative cultural studies were carried out with 51 strains received as identified species of Nocardia, 10 strains designated as Mycobacterium species, and 4 Streptomyces species.

Characteristics that appeared to be stable and of value for group or specific identification were: (*a*) color of the stroma under uniform cultural conditions, (*b*) temperature tolerance, (*c*) hydrolysis of starch, (*d*) liquefaction of gelatin and/or hydrolysis of casein. Characteristics such as paraffin utilization, catalase activity, or indol production appeared to be of little value for identification inasmuch as virtually all strains studied behaved similarly, irrespective of morphologic or other physiologic differences.

Inability to produce a submerged mycelium appeared a useful criterion to distinguish strains of Mycobacterium from markedly fragmenting Nocardia with which they might otherwise be confused.

Production of aerial mycelium and diffusible pigment, degree of fragmentation and acid-fastness, hemolytic and urease activity have only limited value as taxonomic criteria within the genus Nocardia but may be helpful in distinguishing among the genera Mycobacterium, Nocardia, and Streptomyces. *N. intracellularis* did not behave culturally like a Nocardia but appeared more closely related to the genus Mycobacterium.

The strains of Nocardia studied were separated, on the basis of the cultural data presented, into "true" Nocardia and "streptomyces-like" Nocardia. The former have been further tentatively divided into the following groups: (*a*) *N. asteroides* group (all strains of *N. asteroides* plus *N. blackwellii*, *N. cuniculi*, *N. caprae*, *N. sylvodorifera*, *N. polychromogenes*, *N. minima*, and *N. paraffinae*); (*b*) *N. brasiliensis* group (all strains of *N. brasiliensis*); (*c*) *N. corallina* group (*N. corallina; N. globerula*, *N. erythropolis* and *N. convoluta*); and (*d*) *N. opaca group* (*N. opaca* and *N. rubra*).

Sumario

Estudios de la Nocardia y de Otros Actinomycetales: I. Estudios Culturales

Lleváronse a cabo estudios culturales comparados en 51 cepas recibidas como especies identificadas de *Nocardia*, 10 cepas designadas como especies de *Mycobacterium* y 4 especies de *Streptomyces*. Las características que parecieron ser estables y útiles para identificación por grupo o especie fueron: (*a*) color del estroma en condiciones uniformes de cultivo, (*b*) tolerancia a la temperatura, (*c*) hidrólisis del almidón, (*d*) licuación de la gelatina y/o hidrólisis de la caseína.

Características tales como la utilización de parafina, actividad de la catalasa o producción de indol parecieron ser de poco valor para la identificación, dado que virtualmente todas las cepas se comportaron de modo semejante, independientemente de sus diferencias morfológicas o fisiológicas. La incapacidad para producir un micelio sumergido pareció ofrecer una pauta útil para distinguir las cepas de *Mycobacterium* de las nocardias de fragmentación acentuada con las que de otra manera hubieran podido confundirse.

La producción de micelios aéreos y de pigmento difusible, la intensidad de la fragmentación y la ácidorresistencia, la actividad hemolítica y ureásica no poseen más que valor limitado como pautas taxonómicas dentro del género *Nocardia*, pero pueden ser utiles para diferenciar entre los géneros *Mycobacterium*, *Nocardia* y *Streptomyces*. La *N. intracellularis* no se comportó culturalmente como *Nocardia*, sino que pareció ser más afin del género *Mycobacterium*.

A base de los datos culturales presentados, se separaron las cepas de *Nocardia* en nocardias "verdaderas" y nocardias "estreptomicetoideas". Las primeras han sido subdivididas tentativamente en los siguientes grupos: *N. asteroides* (todas las cepas de la *N. asteroides*) y además *N. blackwellii*, *N. cuniculi*, *N. caprae*, *N. sylvodorifera*, *N. polychromogenes*, *N. minima* y *N. paraffinae*; *N. brasiliensis* (todas las cepas del grupo); *N. corallina* (*N. corallina*, *N. globerula*, *N. erytropolis* y *N. convoluta*); y *N. opaca* (*N. opaca* y *N. rubra*).

Resume

Etude de la Nocardia et autres Actinomycetes: I Études culturales.

Des études culturales comparatives ont été effectuées à l'aide de 51 souches fournies comme appartenant à une espèce de *Nocardia* identifiée, 10 souches désignées comme espèce *Mycobacterium* et 4 comme espèce *Streptomyces*. Les caractéristiques apparaissant stables et valables pour une identification spécifique ou de groupe étaient les suivantes: (*a*) couleur du mycélium dans des conditions culturales uniformes, (*b*) stabilité à la chaleur, (*c*) hydrolyse des hydrates de carbone, (*d*) liquéfaction de la gélatine et hydrolyse de la caséine ou l'une ou l'autre de ces réactions.

Les caractéristiques telles que l'utilisation de la paraffine, l'activité de la catalase ou la production d'indol apparaissaient de valeur négligeable pour l'identification, d'autant plus que presque toutes les souches étudiées se comportaient de façon semblable, quelles que soient leurs différences morphologiques ou autres. L'impossibilité d'obtenir un mycélium en profondeur parait un critère utile pour distinguer les souches *Mycobacterium* de celles de la *Nocardia* dont la fragmentation est marquée et avec lesquelles elles pourraient etre confondues.

La production d'un mycélium superficiel et d'un pigment diffusible, le degré de la fragmentation et de l'acido-résistance, l'activité hémolytique et celle de l'uréase n'ont qu'une valeur limitée comme critères taxonomiques à l'égard de la nocardia, mais ils peuvent se révéler utiles pour établir une distinction entre les genres *Mycobacterium*, *Nocardia* et *Streptomyces*. *N. intracellularis* ne se comportait pas en culture comme une *Nocardia*, mais comme apparentée plus étroitement au genre *Mycobacterium*.

Sur la base des données culturales exposées, les souches de *Nocardia* étudiées furent divisées en "vraie" *Nocardia* et *Nocardia "type Streptomyces"*. Les premières ont été

ensuite réparties provisoirement dans les groupes suivants: groupe *N. asteroides* (toutes les souches de *N. asteroides*) plus *N. blackwellii*, *N. cuniculi*, *N. caprea*, *N. sylvodorifera*, *N. polychromogenes*, *N. minima* et *N. paraffinea*; groupe N. brasiliensis (toutes les souches de N. brasiliensis); groupe *N. coralline* (*N. corallina*, *N. globerula N. erythropolis* et *N. convoluta*); et le groupe *N. opaca* (*N. opaca* et *N. rubra*).

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THE USE OF METHYL-METACRILATE IN PATHOLOGIC STUDIES OF THE LUNG[1]

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INTRODUCTION

Recent advances in the field of plastics have enabled the pathologist to use such materials for both research and preparation of museum specimens. Methyl-metacrilate was first applied for this purpose by Romaniak (1) and Patterson (2). Recently a number of writers reported the effective use of this plastic material in preparing museum specimens and corrosions (3–5).

The purpose of embedding is to conserve the specimen and to retain its original shape and color by placing it into a solid material, not fragile, and colorless. In addition, it is required that embedding should cause a favorable change in the properties of the specimen: It should make it transparent and suitable for three-dimensional study and for demonstrating inner structures and relationships previously visible only with the use of a special method.

In the present study a trial has been made by the writers of the use of methyl-metacrilate in preparing pathologic lung specimens. For this purpose, the specimens were prepared before embedding by means of several methods, partly by the application of previously reported techniques and partly by techniques developed by the present writers.

MATERIALS AND METHODS

Fixing: Lobes of human lungs obtained surgically or at autopsy were perfused with carbon dioxide by ligating a cannula into the bronchi and using it to connect the specimens with a bottle containing carbon dioxide. Then the specimens were filled with 10 per cent formalin. During the process of carbon dioxide perfusion, the air in the alveoli of the lungs became replaced by carbon dioxide. The latter was dissolved later in the formalin entering the specimens, thus permitting filling of the lungs without damage (6). In order to obtain a color-retaining specimen, fixing may be carried out by acetone-filling as well.

Drying: After being fixed for four to five days, the specimens were dried by blowing through the bronchi for several days with the help of an air compressor (7). The current of air in the lungs was regulated to obtain a full expansion of the lungs, equivalent to that of the physiologically inflated lungs during inspiration. In this connection, attention should be given to the fact that it is dangerous to dry tuberculous or otherwise infected lungs with compressed air, in view of the danger of dispersing tubercle bacilli. To minimize the risks it is advisable to perform this special method of drying in a room irradiated with ultraviolet rays, and to use a protective garment during the operation.

Another method for drying the acetone-fixed specimens is to cut them into sections 1 to 2 cm. thick and transfer them through an acetone series with gradually increasing concentrations until complete dehydration of the specimen is obtained. The latter method involves shrinking of the specimen to a certain degree. The two methods of drying also may be combined. In this instance air-drying for one to two days is followed by sectioning and dehy-

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drating the specimens with acetone. The dehydrating agent is evaporated from the specimens in the air.

Lead-impregnation method (8, 9): By embedding the dried specimen into a plastic material the parenchyma of the organ and the hollow-system of the part being studied becomes homogeneously filled with that material. The inherent disadvantage of this method is that the specimens become unsuitable for roentgenographic study. In pathologic-anatomic research work it is of interest to compare pathologic sections from the lung with roentgenographic and tomographic films of the patient. Such comparative roentgenographic-pathologic studies may shed some light on the laws governing both roentgenography and tomography. The detection of these laws is greatly facilitated by making the specimens suitable for roentgenography and comparing the films of the specimen with the specimen itself. The lead-impregnation method developed by the present writers renders the specimen embedded in plastics suitable for roentgenographic study. This method is as follows:

After fixation, the entire lung was filled with 40 per cent lead nitrate or, alternatively, the sections of the lung were placed in lead nitrate of 40 per cent. After the lapse of forty-eight hours the specimens saturated with the lead nitrate were washed with tap water until white clouds appeared. The water from the tap, in view of its sulfate content, with the lead nitrate, forms a white lead sulfate precipitate eliminated by the running water from the hollow parts of the organ, such as the lumen of cavities, bronchi, and alveoli, but left in the parenchyma. The lungs prepared by the above method were dried by means of an air compressor or by acetone treatment. The dehydrating agent was then evaporated in the air. To avoid distortion in sections of the lungs during evaporation of the dehydrating agent, it is advisable to place the sections between two sheets of glass.

Polymerizational method. Clearing: The dehydrated specimens were placed into a methyl-metacrilate monomer and kept in a vacuum adjusted to 30 mm. for two days. At intervals of eight hours the vacuum was stopped for a short period of time. The monomer has the property to make the specimen transparent in a manner similar to the clearing agents used generally in histologic technique (xylol, benzol).

Embedding and polymerization: A layer of about 1 cm. of methyl-metacrilate was polymerized into a container made from sheets of glass glued together and corresponding to the final shape of the preparation. Above this layer was placed the material cleared in the monomer. This material was then covered with a prepolymer polymerized to the consistency of honey, until the prepolymer reached a level not only equaling but even exceeding by 1 cm. that of the specimen. During this process, care should be taken to avoid leaving air bubbles in the material. If, however, such bubbles remain they should be eliminated carefully with the help of a vacuum. Then, the preparation was polymerized at low temperature levels. Benzoyl-peroxide of 0.02 to 0.05 per cent was used as a catalyst.

As the result of polymerization, blockpolymer was obtained, which was then removed carefully after eliminating the sheets of glass glued together. The blockpolymer was smoothed to a pleasant shape and finally polished until it became as smooth as ice. In the blockpolymer the specimen retains its original shape and color and may be conserved in a solid, not fragile, state.

Results

Normal lung: When 2 to 3 cm. thick sections from healthy lobes obtained at autopsy are embedded by means of the above method into plastics, the vascular outlines in the lungs can be excellently studied macroscopically, microscopically, or by roentgenographic examination. If drying of the specimen is made without previous fixing with an air compressor, the lungs are red and the vessels can be studied easily, including those a few millimeters in diameter; the capillaries and the erythrocytes in the vessels are also visible (figures 1, 2, and 3).

Placing the cannula in the pulmonary artery of the removed lung and elimi-

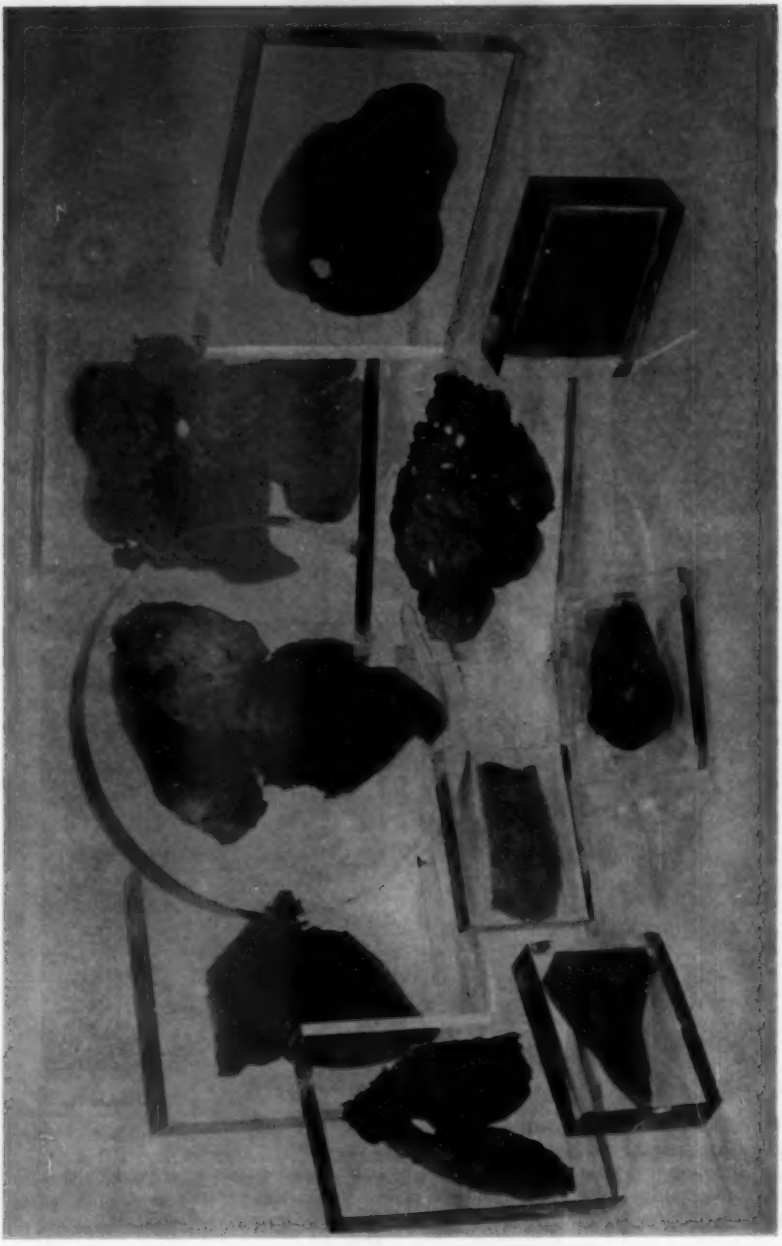


Fig. 1. Areas of lungs embedded into methyl-metacrilate. The circular preparation seen in the midst of this figure has a diameter of 21 to 23 cm.

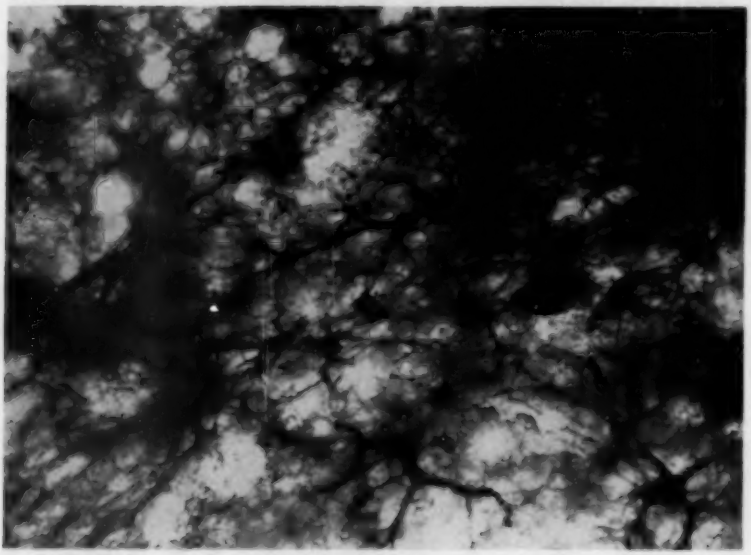


FIG. 2 (Upper). An air-dried, cleared, 1 cm. thick section of a normal lung embedded into methyl-metacrilate. Note the course of the vessels.

FIG. 3 (Lower). Photomicrograph of the vessels of pathologically altered pulmonary areas embedded into plastics. Around the alveoli the vascular system is often intact as opposed to other sites where a deformity of the vascular system is observable.

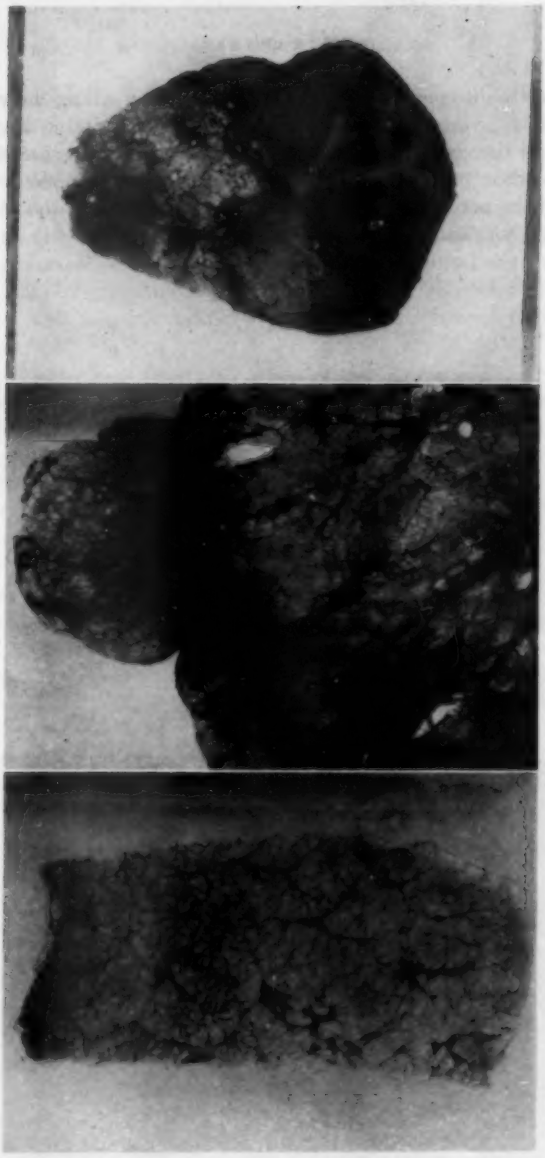
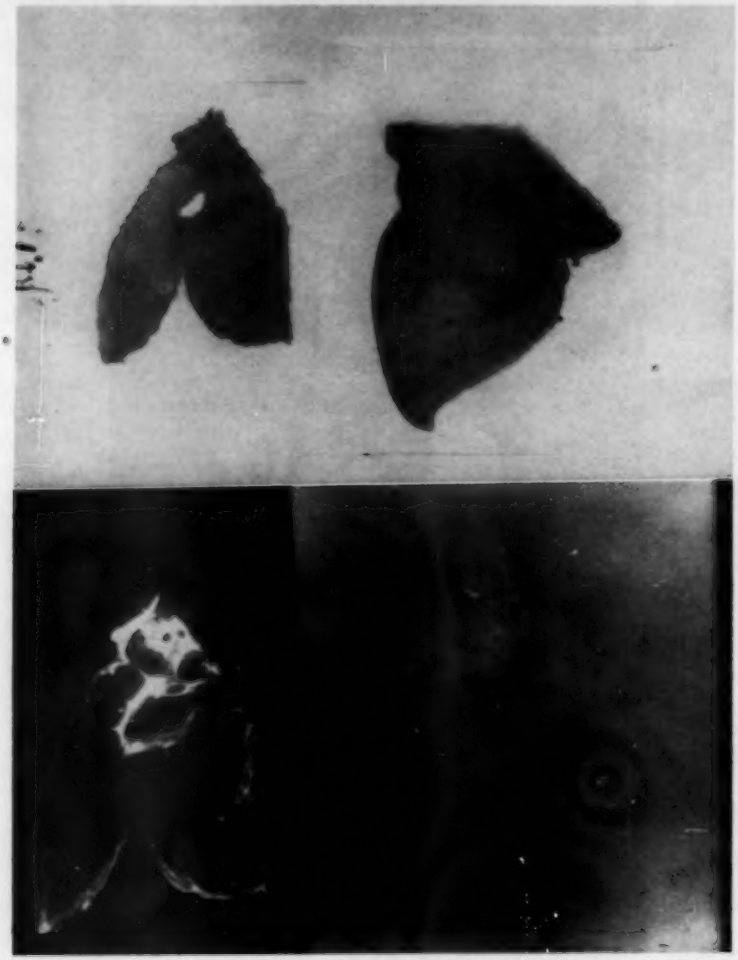


FIG. 4 (Upper). Histologic picture of a section washed through the pulmonary artery, cleared, and embedded into plastics. Well demonstrated are the anthracotic changes located in the perivascular lymphatic gaps.

FIG. 5 (Center). One cm. thick section of lung acetone-fixed, dehydrated, cleared, and embedded into plastics. Note the distribution of anthracosis and fibrosis and the peripheral emphysema.

FIG. 6 (Lower). Apical and subapical tuberculous segments embedded into plastics. In the center of the pulmonary area is shown a cavity transformed into a radiating scar (due to healing and shrinking), from which caseous bronchiectasis leads to the periphery. The pulmonary areas supplied by the closed bronchi are atelectatic and less penetrable to light. The pulmonary areas which are not atelectatic contain localized fibrotic changes.

nating the blood in it completely with running water, the lungs become grayish-white. Following drying and embedding, such lungs are suitable for the study of anthracosis and the connective tissue system of the lungs. In cases in which the collection of carbon particles in the lungs is not too considerable, such particles are located in the peribronchial and perivascular lymphatics, thus outlining the course of these lymphatics in a fairly artificial manner (figure 4). Such prepara-



FIGS. 7 and 8. Two pulmonary areas with cavities polymerized into plastics. The specimen on the left was impregnated with lead; the specimen on the right was left unimpregnated. It may be seen in the roentgenograms that the specimen on the left, in contrast to that on the right, shows rich differences in density of the individual shadows.

tions are very valuable for studying the lymphatic system of the lungs. By exposing experimental animals to the inhalation of pulverized pigments and then preparing the lungs by the above described method, the location of the pigments is readily visible in such preparations.

Fibrosis and emphysema: Fibrosis and emphysema of the lungs, either primary or secondary, can be studied easily on the embedded specimens (figure 5). Provided that the process of clearing is controlled to prevent a complete clearing of the specimen (fibrous areas are less easy to clear), the emphysema is recognizable by increased translucency and dilated alveoli. Fibrotic strands and foci, on the other hand, are characterized by the property that they do not let light through at all.

Tuberculosis: All of the morphologic characteristics of tuberculosis can be well documented on the specimens prepared with the color-retaining method. Cavities are visible to their deeper layers, and the draining bronchus can be found easily. Many of the pathologic changes in the environment of cavities may be well demonstrated as well as the foci situated at the distal subpleural sites of the cavity and the bronchiectatic changes. The relationships between individual foci and cavities and vessels also can be studied easily. It was possible, for example, to demonstrate in one of the specimens the circular course of the vessels around the cavity.

Atelectasis: The extent of atelectasis can be excellently studied on preparations made half transparent in view of the fact that such places are less translucent. Moreover, tuberculous foci and caseous endobronchitis, if present in such areas, are readily visible.

Roentgenographic-pathologic examinations: In figures 7 and 8 two specimens and the corresponding roentgenograms are shown. The specimen on the left is impregnated with lead, whereas the specimen on the right is not impregnated. While the lesions present in the specimen on the left, such as the cavity, the bronchiectasis overlying it, and the peribronchial fibrosis are easily demonstrable on the roentgenogram, the specimen on the right fails to show any structures due to its homogeneous X-ray transmitting property.

Discussion

As compared with other methods used in the pathologic study of the lungs, the described method is associated with different advantages and disadvantages. The advantages are: The specimens embedded into plastics can be well conserved and managed under hygienic conditions (figures 1 and 9). Cleared and embedded thicker specimens are valuable for a three-dimensional study by the pathologist. In addition, this method makes demonstrable relationships which were previously visible only with the help of difficult reconstructions.

By means of a binocular stereomicroscope, the vessels can be examined to their deeper layers. This method, previously applied by Tobin, offers many advantages over those methods of vascular demonstration in which the vessels were made visible by filling with dyes. With the dyes, filling of the vessels is more difficult in the diseased areas because the dyes penetrate more easily intact

FIG. 9. A roentgenogram from the specimen shown in figure 1, above on the right. This specimen contains a cancer with vessels which were filled with radio-opaque substances. The whole specimen was impregnated later with lead and embedded into plastics. In the right part of the preparation the cancer, the bronchiectasis under it, and the absence of vessels in these areas are well shown.

areas which offer less resistance. Also pathologically altered vascular structures and their relationship to pulmonary lesions may be studied easily on the dried and cleared specimens.

Atelectasis, emphysema, and fibrosis can be well demonstrated on thick cleared sections for teaching purposes. A further advantage inherent in this method is that the clearing agent is not a liquid substance but the solid plastic material itself. With the use of our previously described method of lead-impregnation, the specimens could not be conserved for long periods of time after the roentgenograms were obtained, due to gradual loss of the lead content with subsequent drying and distortion of the specimens. By embedding into plastics, lead-impregnated sections remain suitable for roentgenographic study at a later time either for comparison or for any other purposes, including sectional roentgenography.

The disadvantages of the method are: That it is time consuming and difficult to carry out. (However, the transparency of the preparation represents a compensation.) Only dehydrated specimens can be embedded into methyl-metacrilate, and shrinking of certain areas is unavoidable while dehydrating, e.g., areas of caseous necrosis.

Attempts are now being made in this laboratory to polymerize aqueous specimens as well.

Summary

A method of embedding pulmonary specimens into methyl-metacrilate is suggested for pathologic studies of a certain type. With adequate preparation the specimens embedded into plastics are transparent, the vascular structures are visible without the need for injecting them first with dyes, and the location and distribution of anthracosis and fibrosis in the lungs can be easily studied. In specimens made half transparent the extent of atelectasis and of other pathologic changes present in the lungs is suitable for a three-dimensional study. By means of lead-impregnation technique the plastic-embedded specimens become suitable for roentgenography and sectional roentgenography, thus permitting roentgenographic-pathologic examinations, on the one hand, and the study of the laws governing both roentgenography and tomography, on the other hand.

Sumario

El Uso del Metacrilato Metílico en la Histopatología Pulmonar

Propónese para la investigación histopatológica un método que implanta los ejemplares pulmonares en metacrilato de metilo. Con preparación adecuada, los ejemplares incrustados en plásticos son transparentes; las estructuras vasculares quedan visibles sin inyectar antes colorantes; pueden estudiarse fácilmente la localización y la distribución de la antracosis y la fibrosis en los pulmones. En los ejemplares a semitransparencia, la extensión de la atelectasia y de toda otra alteración patológica de los pulmones se presta para un estudio en tres dimensiones. Por medio de la impregnación de plomo en plásticos, los ejemplares implantados se vuelven adecuados para la roentgenografía y la tomografía, permitiendo así, por un lado, hacer exámenes roentgenofotográficos y, por otro, estudiar las leyes que gobiernan tanto la roentgenografía como la tomografía.

Resume

L'emploi du méthacrilate de méthyle en anatomo-pathologie pulmonaire

Une méthode relative à l'enrobage de fragments pulmonaires dans le méthacrilate de méthyle pour l'examen anatomo-pathologique est suggérée. Utilisant une préparation adéquate, les fragments enrobés dans la matière plastique sont transparents; les structures vasculaires sont visibles sans injection préalable de colorants; la localisation et la distribution de l'anthracose et de la sclérose dans les poumons peuvent être étudiées avec facilité. Dans les fragments rendus à moitié transparents, l'importance de l'atélectasie et de toute autre modification pathologique pulmonaire peut être appréciée sous trois dimensions. La radiographie et la tomographie des fragments est rendue possible par l'incorporation de plomb dans la matière plastique, ce qui permet l'examen radiologique des manifestations pathologiques d'une part et l'étude des lois régissant la radiographie et la tomographie d'autre part.

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TUBERCULOUS HILAR AND MEDIASTINAL ADENITIS[1, 2, 3]

Course, Prognosis, and Ambulatory Chemotherapy

D. N. SHIVPURI AND BINDRA BAN

(Received for publication May 16, 1956)

INTRODUCTION

Two hundred and eighty-seven cases of hilar and mediastinal adenitis due to tuberculosis were registered at the Old Delhi Tuberculosis Clinic between January, 1953, and June, 1955. These represented 4.4 per cent of the total number (6,535) of tuberculous patients registered during the same period. Some of the patients presented themselves with symptoms, and others were discovered during family contact examinations. The data from the patients are presented to show several different aspects of their disease, including the effect of ambulatory living conditions with or without antituberculous chemotherapy.

MATERIALS AND METHODS

Many difficulties are encountered in the study of enlarged hilar mediastinal lymph nodes, chiefly due to their inaccessibility for direct biopsy. Every effort was made however, by clinical, roentgenographic, and laboratory examinations (including biopsy of associated cervical or axillary nodes) to exclude such conditions as acute pyogenic infection, paravertebral abscess, lymphoma, sarcoidosis, leukemia, and other causes of adenitis such as measles or pertussis.

The following features were considered in arriving at the diagnosis: (*1*) a history of intimate contact with a tuberculous patient, (*2*) the finding of associated extrapulmonary tuberculosis, (*3*) the tuberculin test, (*4*) the laboratory findings, and (*5*) the chest roentgenogram.

Tuberculin tests (Mantoux) were performed routinely with intermediate-strength PPD-S (0.0001 mg. or 5 TU).

Roentgenograms were taken in 202 cases (71 per cent); postero-anterior views were taken in all of these cases and lateral views in many of them. In 85 cases (29 per cent) only fluoroscopic examinations could be done because of a shortage of X-ray film in the early part of the study. All of the 85 cases were children with positive Mantoux tests, many of whom also had other evidence suggesting a tuberculous etiology, such as an associated lesion in the peripheral lymph nodes or a history of close contact with a tuberculous parent or close associate. However, none of these cases were included in the follow-up studies.

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[1] From the Old Delhi Tuberculosis Clinic and The Vallabhbhai Patel Chest Institute, University of Delhi, India.

[2] Presented in part before the XIII All India Tuberculosis Workers Conference Trivandrum.

[3] The original manuscript, submitted to the REVIEW by Drs. Shivpuri and Ban, was a more detailed presentation of the observations described in the present report. Because of space limitation, it was not possible to publish the manuscript as originally submitted. Accordingly, with the permission of the authors, the original manuscript was turned over to Dr. Thomas Sellers, Jr. of New York Hospital-Cornell Medical Center, New York, New York, for condensation. The authors of the manuscript and the editor of the REVIEW wish to express their appreciation to Dr. Sellers for his very considerable contribution to the publication of this report.

Initial Classification

All cases followed were roentgenographically classified into three groups by the size of the enlarged mediastinal nodes, as measured by the transverse diameter of the superior mediastinum. All skiagrams were taken in the erect position at a distance of 6 feet with the exception of children too young to stand erect. For purposes of the classification the following criteria were used:

Marked enlargement: Those with a transverse diameter of the mediastinum greater than 5.5 cm.

Moderate enlargement: Those with a transverse diameter between 4.5 and 5.5 cm.

Slight enlargement: Those with a transverse diameter of 4 to 4.5 cm.

Follow-up Studies

For the purposes of follow-up observations, the patients fell into two groups: those who received no chemotherapy and those who were on ambulatory chemotherapy for a period of time. "Ambulatory chemotherapy" means that the patient, although on antituberculous chemotherapy, did not take absolute rest in bed during the illness and, as soon as his symptoms were relieved, started leading a relatively normal life, avoiding only strenuous exertion.

Follow-up classification was based on a comparison of the initial roentgenogram with subsequent ones, under the headings of *worse*, *stationary*, or *improved*.

Worse meant either an increase in the size of the lymph nodes or the appearance of a new lesion in the lung. *Stationary* meant that there was no change in the size of the lymph nodes or the transverse diameter of the mediastinum although the patients were symptomatically improved. Improvement was graded according to the decrease noted in the transverse mediastinal diameter as follows:

Marked improvement: reduction of the transverse diameter by 1 cm. or more

Moderate improvement: reduction of the transverse diameter by 0.5 to 1 cm.

Slight improvement: reduction of the transverse diameter by less than 0.5 cm.

Results

The age and sex incidence for the series of 287 cases is shown in figure 1 and table 1. Certain interesting features are noted. Although the patients less than ten years of age are almost equally divided between the sexes, the number of cases in females rises sharply during the next ten years, whereas the incidence among males falls off rapidly during the same period. No cases were encountered among males after the age of twenty. Although there were 19 per cent more males than females among all tuberculous cases registered, cases of mediastinal adenitis were 2.8 times more common among the females than among the males (6.8 per cent to 2.4 per cent).

Associated tuberculous lesions were present in 200 (70 per cent) of the 287 patients (74.4 per cent of females and 59 per cent of males). The incidence of these lesions by site is shown in table 2. It is of interest that cervical and axillary adenitis were relatively more common among males while pleuropulmonary types predominated among females.

A history of close contact with a tuberculous parent or other relative was elicited in 56 females and 38 males who represent about one-third of the total. In 75 patients from whom detailed information is available, the tuberculous contact was the mother in 32 per cent, the father in 17 per cent, a sister in 27 per cent, and a brother in 11 per cent.

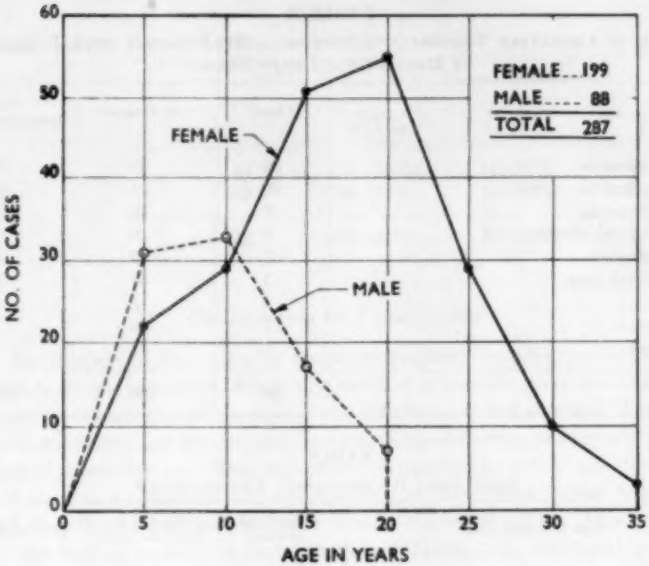

FIG. 1. Graph showing the age incidence of mediastinal adenitis.

TABLE 1

AGE AND SEX INCIDENCE OF PATIENTS WITH TUBERCULOUS HILAR AND MEDIASTINAL ADENITIS

Age	Males	Females	Total
years			
1–5	31 (35.2%)	22 (11%)	53 (18.5%)
6–10	33 (37.5%)	29 (14.6%)	62 (21.6%)
11–15	17 (19.3%)	51 (25.6%)	68 (23.7%)
16–20	7 (8%)	55 (27.6%)	62 (21.6%)
21–25		29 (14.6%)	29 (10.1%)
26–30		10 (5.0%)	10 (3.5%)
31–35		3 (1.5%)	3 (1%)
Total	88 (100%)	199 (100%)	287 (100%)

The tuberculin test was positive in all patients tested, a total of 243. In the remaining 44 patients who were not tested, other important evidence of tuberculosis was encountered, such as associated tuberculous lesions in 36 cases, a history of close contact with an infectious case of tuberculosis in 12 cases, and the demonstration of tubercle bacilli by sputum or gastric cultures of 4 patients.

The uniform finding of tuberculin sensitivity reflected the early stage of the primary complex that was represented by hilar and mediastinal enlargement. In some patients with slight enlargement of the mediastinum (4 to 4.5 cm. in

TABLE 2

INCIDENCE OF ASSOCIATED TUBERCULOUS LESIONS IN 200 PATIENTS WITH TUBERCULOSIS OF MEDIASTINAL LYMPH NODES

	52 Males	148 Females	Total Associated Lesions
Cervical adenitis	26	59	85
Axillary adenitis	16	28	44
Intra-abdominal	4	16	20
Bone and joint disease	1	6	7
Pleural effusion	1	19	20
Parenchymal lung	5	54	59
Miliary	2	5	7
Cold abscess	1	2	3
Pericarditis	0	1	1
Total	56	190	246

TABLE 3

RESULTS OF BACTERIOLOGIC EXAMINATIONS

Type of Examination	Total Number of Cases	Positive for Tubercle Bacilli	Per Cent Positive
Sputum direct smear	30	10	33.3
Sputum culture	4	1	25.0
Laryngeal swab culture	26	4	15.4
Gastric lavage culture	38	13	34.0
Pericardial fluid culture	1	1	100.0
Associated lymph node biopsy culture	5	5	100.0
Total	104	34	32.7

diameter) no reaction to 5 tuberculin units of PPD was observed. A positive reaction was obtained in all of these patients by a 1:100 dilution (1.0 mg.) of Old Tuberculin (OT). Although none of the patients who reacted only to OT were included in the report, it is noteworthy that this group required approximately the equivalent of 250 TU of PPD for a positive reaction. It is believed that this was the consequence of a longer duration of tuberculous disease, which possibly had already passed the early phase of highest allergy.

Examination for tubercle bacilli was performed in 104 cases, and the results were positive in 34 (table 3). The most satisfactory procedure from a bacteriologic standpoint seemed to be culture from biopsy of an associated lymph node, but this was done in only 5 instances. Gastric lavage culture was positive in one-third of the cases as was direct smear of unconcentrated sputum. In every patient with acid-fast bacilli in the sputum there was an associated parenchymal lesion. Although biopsy of associated enlarged lymph nodes was done in only 10 cases, all showed the classical histopathologic features of tuberculosis.

TABLE 4

ROENTGENOGRAPHIC CLASSIFICATION OF INITIAL CONDITION IN MALES AND FEMALES

	Total	Degree of Enlargement		
		Slight 4–4.5 cm.	Moderate 4.5–5.5 cm.	Marked More than 5.5 cm.
Males	34	13 (38%)	12 (35%)	9 (26%)
Females	102	35 (34%)	51 (50%)	16 (16%)
Total	136	48 (35%)	63 (46%)	25 (18%)

Roentgenographic Classification

The distribution of the cases by roentgenographic classification of the initial condition is shown in table 4. When this method of classification was improvised, difficulties were anticipated because of the difference in the size and shape of the chest in the different age groups and because of the difficulty in properly positioning small children for the chest skiagrams. However, in actual practice, it was found that even in children the enlargement of the lymph nodes was so pronounced that the mediastinal diameter usually exceeded 4.5 cm. This point may explain the finding in table 4 that the male patients, who belonged in a much younger age group than the female patients, had greater percentage of incidence of marked mediastinal enlargement; that is, a transverse diameter of more than 5.5 cm.

It is interesting that in 89 cases (30 per cent) the enlargement of the hilar nodes, as seen in the posterio-anterior roentgenograms, was more marked and conspicuous than that of the mediastinal nodes.

Effects on Bronchi

In this series of hilar and mediastinal adenitis, complete occlusion of the bronchus, as indicated by atelectasis of the related segment, was present in 9 per cent of the total (or 7.5 per cent of the females and 11 per cent of the males). This difference, although not statistically significant, may again be due to the younger age of the male patients, when the bronchi tend to be softer and smaller in caliber and the enlargement of the lymph nodes most pronounced.

The lobar distribution of atelectasis is presented in table 5. Most frequently involved was the right upper lobe, affected in almost half of the cases. Seventy-three per cent of the total showed involvement of a bronchus on the right side.

The findings are in general agreement with those of Head and Moen (1) who found that the right middle lobe in their series stood third in frequency of involvement.

With regard to the high frequency of right upper lobe involvement, it is interesting to note that Jeune and associates (2), reporting bronchoscopic studies in primary tuberculosis, found stenosis of that bronchus in 83 per cent of their

TABLE 5

INCIDENCE OF ATELECTASIS BY LOBES*

	Right	Left
Upper	12 (46%)	6 (23%)
Middle	1 (4%)	
Lower	6 (23%)	1 (4%)
Total	73%	27%

* No patient had more than one atelectatic lobe.

cases. They explain this by the fact that the interbronchial node adjacent to the right upper lobe bronchus receives lymphatic drainage from the entire right lung.

Chemotherapy

Of the 287 patients, 136 patients (47 per cent) were followed for a period ranging from less than three months to five years. Five year follow-up observations were available in nearly a dozen patients who had roentgenograms antedating January, 1953. The average period of follow-up observation was fourteen months, including observation of 3 patients in the group without chemotherapy who died within three months. The duration of follow-up study in the groups with and without antimicrobial therapy is given in table 6. Forty-six patients received no antituberculous drugs while 85 received some combination of antimicrobial agents for a period varying from four to thirty-two weeks, as shown in table 7. (In addition there were 5 patients who did not receive chemotherapy for the first four to six months; when they later became worse and developed complications, drug therapy was begun. These have been included in table 6 among those who received chemotherapy, but have been omitted in tables 4, 7, 8, and 9.) Fifty-one patients received PAS and isoniazid, 36 were given streptomycin and isoniazid, and 3 received streptomycin and PAS. The dosage used was isoniazid, 3 mg. per kg. daily; streptomycin, 20 to 25 mg. per kg. twice a week; and PAS, 150 to 170 mg. per kg. daily.

The results of ambulatory chemotherapy for variable periods of drug administration are presented in table 7. As might be expected, the longer the period of chemotherapy, the fewer patients who were *worse*, *stationary*, or only *slightly improved*, and the more patients who showed *moderate* or *marked improvement*. A lag period of several months was always noted between the clinical improvement of the patient and the discovery of any reduction in the size of the mediastinum.

Ten of the patients with atelectatic segments did not receive chemotherapy. Six of these were lost to follow-up study, but within one year all of the remaining 4 had become worse with extension of disease and persistence of atelectasis. Of the 16 who received chemotherapy, 8 patients were treated for an average of six months, and the lymph node disease cleared in all of them. One lesion cleared in eight months but, in the other cases, twelve to fifteen months elapsed after the

TABLE 6

PERIOD OF FOLLOW-UP OBSERVATIONS OF TWO GROUPS OF HILAR AND MEDIASTINAL ADENITIS

Period of Follow-up Observations	Total	3 Months	3-6 Months	6-12 Months	1-2 Years	2-3 Years	3-4 Years	4-5 Years
Without chemotherapy	46	3	17	14	6	2	3	1
With ambulatory chemotherapy	90	—	22	20	25	8	3	3
Total	136	3	39	43	31	10	6	4

TABLE 7

FINAL ROENTGENOGRAPHIC CLASSIFICATION OF MEDIASTINAL LYMPH NODES IN RELATION TO THE PERIOD OF THE CHEMOTHERAPY

Period of Chemotherapy	Number of Cases	Dead	Worse	Stationary or Slight Improvement	Moderate or Marked Improvement
months					
1-2	24	0	4 (16.7%)	15 (62.5%)	5 (20.8%)
2-4	34	0	0	20 (58.8%)	14 (41.2%)
4-6	16	0	0	7 (43.8%)	9 (56.2%)
6-8	11	0	0	4 (36.4%)	7 (63.6%)
Total	85	0	4 (4.7%)	46 (54.1%)	35 (41.2%)

TABLE 8

INITIAL ROENTGENOGRAPHIC CLASSIFICATION OF THE GROUPS WITH AND WITHOUT CHEMOTHERAPY

	Total Cases	Slight Enlargement Tr. D.* 4 to 4.5 cm.	Moderate Enlargement Tr. D. 4.5 to 5.5 cm.	Marked Enlargement Tr. D. more than 5.5 cm.
Without chemotherapy	46	9 (19.6%)	26 (56.5%)	11 (23.9%)
With ambulatory chemotherapy	85	16 (18.8%)	37 (43.5%)	32 (37.6%)

* Tr. D. = Transverse diameter

TABLE 9

ROENTGENOGRAPHIC CLASSIFICATION OF FINAL CONDITION

	Total	Dead	Worse	Stationary	Slight Improvement up to 0.5 cm.	Moderate Improvement up to 0.5 to 1 cm.	Marked Improvement, more than 1.0 cm.
Without chemotherapy	46	5 (10.9%)	12 (26.1%)	16 (34.8%)	9 (19.6%)	2 (4.3%)	2 (4.3%)
With ambulatory chemotherapy	85	—	4 (4.7%)	17 (20%)	29 (34.1%)	17 (20%)	18.2 (21%)

beginning of chemotherapy before resolution occurred. In another group of 6 patients who received chemotherapy for an average of three and one-half months, a five-month follow-up observation revealed persistent atelectasis. In 2 other patients treated for two and five months, respectively, atelectasis was still present two years later. Despite the limitations of follow-up observation, it seems clear that, even after chemotherapy for six months, the atelectasis due to mediastinal and hilar adenopathy usually takes more than twelve months to disappear.

Although initially (table 8) the total percentage of marked and moderate mediastinal lymph node enlargement was similar in both the treated (80 per cent) and the control groups (81 per cent), the follow-up observations (table 9) revealed that 10.9 per cent of the control group were dead and 26.1 per cent were worse. On the other hand, of the group treated with ambulatory chemotherapy, none had died and only 4.7 per cent were worse. Furthermore, all of those who were worse had received chemotherapy for only four to six weeks before giving it up of their own accord.

Also it may be seen that only 8.6 per cent of the group without chemotherapy showed *marked* or *moderate improvement* in the mediastinal lymph nodes. In contrast, 41.2 per cent of the group with ambulatory chemotherapy showed *significant improvement.*

It should be brought out that the *stationary* group was largely composed of those initially classified as having slight enlargement of the mediastinal shadow. The infections were probably of longer duration, having passed the peak of tuberculous activity, and the lesions may have become partially stabilized. Although these patients initially had some symptoms suggesting disease activity, they invariably fell into the group with only slight enlargement of the mediastinum. From this information one can predict that persons showing slight mediastinal enlargement with irregular symptoms of long duration will most likely continue to present *stationary* roentgenographic findings. This may be because minor and minute changes in the mediastinum are difficult to detect by roentgenography even though there is clinical improvement. Moreover, it is difficult to measure reduction in size of lymph nodes more centrally placed than the hilar or paratracheal nodes, i.e., the bifurcation group.

Discussion

The greater incidence of tuberculous hilar and mediastinal adenitis among females more than ten years of age is striking and strongly suggests that factors of decreased host resistance are involved. In females the onset of puberty is much earlier than in males. They are exposed to the effect of hormonal changes and other stressful processes of puberty and child bearing. For these or other reasons, females seem unable to handle the infection as effectively as do the pre-pubertal males of the same chronologic age. This observation is further supported by the fact that the incidence of associated tuberculous lesions was much greater among the females. The incidence of tuberculous hilar and mediastinal adenitis in female patients was 2.8 times that of the male. Yet among females the inci-

dence of pleural effusion was 19 times that of the male; pulmonary parenchymal lesions were 11 times, bone and joint lesions 6 times, and abdominal tuberculosis 4 times more common in females than in males (table 2).

These data indicate that in Old Delhi the chances for female children and adolescents to develop clinical tuberculosis and its complications are greater than for male children, who usually are first infected at a prepubertal age. The approximate incidence of clinical disease among the tuberculin-positive population is quoted at 1.6 per cent (3). In this area the rate among adolescent and young adult females may be more than this, possibly even 2 to 3 per cent.

Complications following gross tuberculous lesions in the hilar and mediastinal lymph nodes are more likely to occur than the complications resulting from the primary pulmonary focus. The constant relationship of these nodes to the bronchial tree, mediastinal structures, and pulmonary vessels, and the clinical importance thereof, has been demonstrated by several workers (1, 3–5, 7, 8).

Large caseating lymph node masses immediately adjacent to the main bronchi and their branches may cause pressure and occlusion of these bronchi, leading to atelectasis of the corresponding segment. Also the caseating nodes may ulcerate through the bronchial wall, giving rise to severe hemorrhage and tuberculous endobronchitis as well as to tuberculous aspiration pneumonia (5, 9–12). Bronchoscopic studies reported by some workers have revealed bronchostenosis in more than 30 per cent of all cases of primary tuberculosis in children (2).

In many cases the collapsed part of the lung re-expands when the lymph node lesion regresses and the bronchial lumen becomes patent. This is necessarily a very slow process which may take one to three years. Sometimes re-expansion does not occur, particularly when ulceration takes place through the wall of the bronchus followed by healing with bronchostenosis. At times a calcified enlarged lymph node maintains extrinsic pressure, and permanent bronchostenosis ensues. These patients eventually develop suppurative diseases of the bronchi and lungs, particularly bronchiectasis (3, 4, 13, 14). The entity described as the "middle-lobe syndrome" (4, 14, and 15) is a well-known representative of this phenomenon.

A complacent attitude seems to exist toward the roentgenographically manifest lymph node component of a primary complex, with the attitude among many that chemotherapy is not indicated. The results of the follow-up study of the two groups as given in table 9, however, would seem to emphasize the seriousness of this type of infection and point up the value of antituberculous chemotherapy for a prolonged period, probably at least one year. Based on this study, the outlook for untreated cases of tuberculous mediastinal adenitis showing moderate to marked lymph node enlargement is such that within two and one-half years more than one-third will be worse (26 per cent) or dead (11 per cent).

The disease in more than half (54per cent) will remain stationary or show only slight improvement. In contrast, even though 68 per cent of the patients in the present study received ambulatory chemotherapy for less than four months (table 7), the improved prognosis in the treated group is quite apparent. Furthermore, it will be noted that the longer the duration of chemotherapy the greater the percentage of patients showing significant roentgenographic improvement.

These data lead one to the belief that prolonged and uninterrupted drug therapy would achieve still more favorable results.

The fact that the patients receiving chemotherapy did not observe absolute bed rest and lived like normal persons after relief of symptoms is a point worth much emphasis. It shows that this form of tuberculosis can be treated effectively even with ambulatory chemotherapy.

Not every tuberculous primary complex develops roentgenographically significant enlargement of the regional lymph nodes, even in children. It appears that such enlargement of the lymph node component of the primary complex is indicative of factors unfavorable for the patient, such as low resistance of the host or high dosage and virulence of the infecting organism. The presence of these factors is a further indication for chemotherapy for more than one year.

Summary

Two hundred and eighty-seven cases of hilar and mediastinal adenitis due to tuberculosis were studied over a thirty-month period in Old Delhi, India. The incidence in females was 2.3 times that of the males, and the maximal incidence occurred at a later age (ten to twenty years) among females than among males (three to ten years).

A positive tuberculin reaction was obtained in 100 per cent of the patients tested. Tubercle bacilli were demonstrated in 23 per cent and a history of contact with active tuberculosis was obtained in 33 per cent of the patients.

Associated tuberculous lesions were present in about 75 per cent of females and 60 per cent of males. Such complications as pleural effusion or parenchymal lesion were more common in females, while cervical and axillary adenitis predominated in the males. Atelectasis (segmental or lobar) was detected in 9 per cent of the patients and was most common in the right upper lobe.

One hundred and thirty-six patients (47 per cent) were followed for a period of three months to five years (average period, fourteen months). One-third of these received no chemotherapy; the remainder received chemotherapy for varying periods ranging from four to thirty-two weeks.

Marked or moderate enlargement of the mediastinal lymph nodes was present in about 80 per cent of both treated and untreated groups, but significant improvement was noted in only 8.6 per cent of the untreated group as opposed to 41.2 per cent of the ambulatory chemotherapy group.

Ambulatory chemotherapy proved to be practical in this situation. The value of prolonged antituberculous chemotherapy was stressed by the finding that improvement became more marked, while unsatisfactory results became less frequent, the longer chemotherapy was continued. Chemotherapy for at least one year would seem to be indicated for all cases of tuberculous hilar and mediastinal adenitis.

Sumario

Adenitis Hiliar y Mediastínica Tuberculosa: Evolución, Pronóstico y Quimioterapia en Enfermos Ambulantes

Doscientos y ochenta siete casos de adenitis hiliar y mediastínica debida a tuberculosis fueron estudiados durante un período de treinta meses en el Viejo Delhi, India. La incidencia en las mujeres fué 2.3 veces mayor que en los varones y la incidencia máxima tuvo lugar a una edad mayor (de los diez a los veinte años) entre las mujeres que en los varones (de los tres a los diez años).

En 100 por ciento de los enfermos comprobados, se obtuvo una reacción positiva a la tuberculina. Se encontraron bacilos tuberculosos en 23 por ciento y había historia de contacto con tuberculosis activa en 33 por ciento de los enfermos.

Aproximadamente en 75 por ciento de las mujeres y 60 por ciento de los varones había lesiones tuberculosas asociadas. Complicaciones tales como derrame pleural o lesión parenquimatosa fueron más comunes en las mujeres, mientras que la adenitis cervical y axilar predominó en los varones. Se descubrió atelectasia (segmental o lobular) en 9 por ciento de los enfermos, siendo más común en el lóbulo superior derecho.

Se mantuvo en observación a 136 enfermos (47 por ciento) por un período de tres meses a cinco años (promedio, catorce meses). La tercera parte de éstos no recibió quimioterapia; el resto la recibió durante períodos que variaron de cuatro a treinta y dos semanas.

Había hipertrofia notable o moderada de los ganglios linfáticos del mediastino aproximadamente en 80 por ciento de los grupos tratado y sin tratar, pero no se notó mejoría de importancia más que en 8.6 por ciento del grupo sin tratar, comparado con 41,2 por ciento en el grupo de quimioterapia a base ambulante.

La quimioterapia a base ambulante resultó práctica en esta situación. Recalcó el valor de la quimioterapia antituberculosa prolongada el hallazgo de que, mientras más tiempo duraba, más notable era la mejoría en tanto que se volvían menos frecuentes los resultados poco satisfactorios. La quimioterapia a lo menos por un año parece indicada en todo caso de adenitis hiliar y mediastínica tuberculosa.

Resume

Adénite tuberculeuse hilaire et médiastinale, pronostic et chimiothérapie ambulatoire

Deux cents quatre-vingt-sept cas d'adénites hilaires et médiastinales dus à la tuberculose ont été étudiés pendant une période de trente mois à Old Delhi, Inde. Chez les femmes l'incidence 2, 3 fois plus élevée que celle observée chez l'homme et la fréquence maxima se produisait à un âge plus avancé (10 à 20 ans) chez les femmes que parmi les hommes (3 à 10 ans).

Une réaction positive à la tuberculine fut obtenue chez 100% des malades soumis au test. Des bacilles tuberculeux furent isolés dans 23% des cas; les antécédents indiquaient un contact avec une tuberculose en activité chez 33% des patients.

Des lésions tuberculeuses associées étaient présentes chez 75% des femmes et 60% des hommes. Les complications telles que l'épanchement pleural ou les lésions du parenchyme étaient plus fréquentes chez les femmes, tandis que les adénites cervicales et axillaires prédominaient chez les hommes. L'atélectasie (segmentaire ou lobaire) fut découverte chez 9% des patients, elle était plus fréquente dans le lobe supérieur droit.

Cent trente-six patients (47%) furent suivis pendant une période de trois mois à cinq ans (période moyenne, 14 mois). Un tiers d'entre eux ne reçurent aucune chimiothérapie; les autres furent soumis à la chimiothérapie pendant des périodes diverses allant de quatre à trente-deux semaines.

L'hypertrophie marquée ou modérée des ganglions lymphatiques médiastinaux était présente dans environ 80% des cas dans les groupes traités ou non traités, mais une amélioration significative n'a été observée que dans 8, 6% des cas seulement dans le groupe non traité par comparaison avec les 41, 2% du groupe traité par la chimiothérapie ambulatoire.

La chimiothérapie ambulatoire a démontré dans ces conditions son caractère pratique. La valeur de la chimiothérapie antituberculeuse prolongée a été mise en évidence par la découverte que l'amélioration devenait plus marquée en meme temps que les résultats défavorables devenaient moins fréquents à mesure que la chimiothérapie prolongée était poursuivie. La chimiothérapie pratiquée pendant au moins une année semble indiquée dans tous les cas d'adénite tuberculeuse hilaire et médiastinale.

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THE CURRENT STATUS AND TREATMENT OF LYMPHATIC TUBERCULOSIS[1]

A Review Based on the Experience with 120 Cases

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INTRODUCTION AND GENERAL CONSIDERATIONS

Historically, tuberculous involvement of the lymphatic system is one of the most ancient of described diseases. The first references were by the ancient Greeks. In later centuries, it was the widely prevalent "King's Evil" or scrofula. In more recent times, the incidence of lymph node tuberculosis has shown a steady decline in many parts of the world.

In any discussion of lymphatic tuberculosis, it is most essential to specify exactly what one means by the term. In a general sense, all tuberculosis implies lymphatic involvement. In the primary infection, there is always regional lymph node involvement of areas draining the primary focus of infection. These nodes may vary widely in the type and degree of their involvement, and the pathologic picture may range from that of reactive hyperplasia to complete caseation and necrosis.

Following the primary infection, there is frequently, if not usually, a phase of postprimary hematogenous dissemination of tubercle bacilli. These organisms are filtered out in the reticuloendothelial system including, of course, the lymph nodes. While this phase may never become clinically apparent or significant, there is, nevertheless, tuberculous involvement of the lymphatic system. In any of the clinically significant forms of diffuse hematogenous tuberculosis, the lymph nodes are necessarily involved.

At any point following the postprimary hematogenous dissemination, a lymph node or group of nodes might break down with progressive disease, giving rise to clinically significant lymphatic tuberculosis. This may occur almost immediately, the primary infection progressing directly into the various manifestations of lymphatic tuberculosis. On the other hand, the lymphatic foci may remain dormant for years, only to break down and give rise to significant lymphatic tuberculosis at any time in the life of the individual. In fact, one of the patients in the present series was eighty-one years old at the time his tuberculous adenitis was found to be active.

Thus, while lymphatic tuberculosis is a postprimary manifestation, it may not become apparent for a number of years following the primary infection. In the majority of the cases, however, the breakdown is believed to be relatively early, usually within the first few years following the primary infection.

Lymphatic tuberculosis, therefore, usually first manifests itself in childhood in those areas of the world in which the childhood infection rate is high because of a high prevalence of tuberculous infection in the locality. Conversely, in those

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parts of the world in which the reservoir of infection is low and the incidence of tuberculin conversion—and therefore infection— is low, primary infections tend to be acquired at a later date. In these circumstances, lymphatic tuberculosis, when it occurs, tends to be seen first in the young adult, or adult age groups, although, as mentioned previously, lymphatic tuberculosis can be seen first in any locality at any age.

It has been well demonstrated in past years that in many parts of the world lymphatic tuberculosis was to a large extent a manifestation of bovine infection. With the advent of tuberculin testing of herds and pasteurization of milk, the incidence of lymphatic tuberculosis in these countries fell off markedly. However, this is not the whole answer since lymphatic tuberculosis occurs among people who have never had any history of raw milk ingestion and is probably due to the prevalence of a large reservoir of human infection. The control of bovine infection has certainly been responsible for a lessening of the amount of lymphatic tuberculosis in many areas of the world, but it is obviously not the whole answer to the problem.

Although much has been written on this subject, very little has appeared in the past few years summarizing any large series of cases treated with modern chemotherapy. It is the purpose of this paper to report the experience with a series of 120 patients treated recently at a major tuberculosis center in the United States. The present series is based on patients admitted to the adult tuberculosis service at Fitzsimons Army Hospital and specifically excludes pediatric cases. The age range of the patients is from fourteen to eighty-one years (table 1). Of the 120 patients, 115 were in the seventeen to forty age group and eighty-nine of these were between the ages of twenty-one and thirty. There were ninety-nine males and twenty-one females in the series. This age–sex distribution is probably explained by the fact that this study is from a large army hospital with a preponderantly young male patient load.

Included in the series are only those patients who demonstrated clinically significant adenopathy considered to represent active lymph node tuberculosis. All cases were excluded in which incidental calcified nodes were noted on

TABLE 1

AGE DISTRIBUTION OF 120 PATIENTS WITH LYMPHATIC TUBERCULOSIS

Age	Number of Patients
years	
14	1
15–20	13
21–30	89
31–40	13
41–50	3
81	1
	120

roentgenography or in which clinically insignificant or inactive lymph node involvement was demonstrable.

Incidence and Types of Involvement

Using the above criteria, 120 cases of significant active lymph node tuberculosis were found among 3,958 cases of active tuberculosis on the adult tuberculosis service at Fitzsimons Army Hospital during the period from January 1, 1952, through December 31, 1955. The over-all incidence of lymph node tuberculosis for this period was 3.03 per cent of all cases of active tuberculosis.

Ethnic distribution: Fifty-five of the cases (45.8 per cent) occurred in U. S. Negroes, 32 (26.7 per cent) in U. S. whites, and 6 in U. S. Indians. The remaining 33 patients (27.5 per cent) were divided as follows: 18 patients were of Spanish-American origin; 8 were Oriental; and one patient was from the Republic of India (table 2*A*).

It might be argued that hereditary factors play no role in the racial development of lymphatic tuberculosis but that these subgroups with higher incidence rates merely reflect environmental differences. In other words, the groups with the higher prevalence of lymph node disease simply represent cultural groups with a higher incidence of tuberculosis prevalence and exposure in general. On the other hand, the population observed in an Army hospital represents a fairly

TABLE 2*A*

Distribution by Sex and Ethnic Group of 120 Patients with Lymphatic Tuberculosis

Race	Sex		Total	Per Cent
	Male	Female		
U.S. white	26	6	32	26.67
U.S. Negro	49	6	55	45.83
Oriental	5	3	8	
Spanish-American	14	4	18	
U.S. Indian	4	2	6	27.50
Indian	1	0	1	
	99	21	120	100.00

TABLE 2*B*

Distribution by Race or Ethnic Group of All Cases of Active Tuberculosis Discharged in 1955 (Including 17 Deaths)
(Included for Purpose of Comparison with Table 2*A*)

Race	Number	Per Cent
U. S. white	572	78.04
U. S. Negro	95	12.96
Other	66	9.00
	733	100.00

good cross section of racial, ethnic, and socio-economic groups as seen in the United States today. If the environmental argument is wholly valid, there should be similar differences between the composition of the military group as a whole and the racial or ethnic distribution of all types of active tuberculosis as observed in a military hospital.

In table 2*B* the ethnic distribution of 733 proved active tuberculous infections from this hospital in the year 1955 is presented. It may be seen, for example, that 78 per cent of these patients were U. S. whites and 13 per cent were U. S. Negroes. This distribution of active cases of tuberculosis of all types is very much in line with the white–non-white distribution of the general military population. The fact that the distribution of lymphatic tuberculosis is so vastly different from the over-all distribution of all types of tuberculosis as seen at this hospital might constitute evidence that the increased incidence of lymphatic tuberculosis is not merely a reflection of an increased incidence of tuberculosis in general among certain groups, but that certain genetic factors must be involved.

Lymph nodes: A study of the lymph node groups involved proves to be of some interest (table 3). The most frequently involved groups of nodes are the cervical groups. These are probably affected as a result of postprimary lymphatic spread or by hematogenous dissemination from an original focus in the lung. In the older literature, mention was made of the role of the tonsil as a portal of entry for later cervical lymph node involvement. However, in only one of the cases in the present series was there any proved tonsillar involvement. While it is impossible to deny at times that the tonsil could be the portal of entry for cervical tuberculosis, it is most difficult to believe that this mechanism plays any major role in the pathogenesis of cervical lymphadenitis.

TABLE 3

DISTRIBUTION OF 120 CASES OF TUBERCULOUS LYMPHADENITIS BY LYMPH NODE GROUPS INVOLVED

Anterior cervical	27
Posterior cervical	17
All or unspecified cervical	23
Mediastinal	24
Cervical and mediastinal	9
Axillary	7
Cervical and axillary	4
Anterior cervical, axillary, and mediastinal	1
Anterior cervical and tonsil	1
Axillary and mediastinal	1
Mediastinal and inguinal	1
Mesenteric	1
Inguinal	1
Epitrochlear	1
None on original admission—axillary relapse	1
None on admission; mediastinal found at surgery in August, 1953; anterior cervical in January, 1954	1
	120

In older series of cases, a relatively high incidence of mesenteric node involvement was always described. It should be mentioned that the diagnosis of mesenteric adenitis usually depends on symptoms of considerable severity leading to diagnosis at laparotomy. Consequently, if the nodes were producing relatively few symptoms, they could easily be overlooked. However, in the entire series, only one case of involvement of the mesenteric nodes was found. This could be explained most easily by the decrease in milk-borne bovine tuberculous infections in which the gastrointestinal tract and the regional mesenteric nodes are the site of the primary infection. Another factor which might explain the paucity of mesenteric node involvement is that, with the advent of chemotherapy, there has been a great decline in contiguous spread of open pulmonary lesions, with a consequent drop in the incidence of intestinal tuberculosis in general and, therefore, of secondary mesenteric node involvement in particular.

In the present series, mediastinal node involvement was second in frequency only to cervical node involvement. This incidence is proportionately greater than in previously reported studies in the adult. Mediastinal tuberculosis in the adult may well be an expression of a primary pulmonary infection and might indicate the increasing importance of primary tuberculosis in the adult group. However, the previous status of the tuberculin reaction was not available for most of the patients and it was not possible, therefore, to prove how much of the mediastinal node involvement in the present series actually represented a primary infection.

Marfan's Law: In reviewing the older literature, one receives the impression that lymphatic tuberculosis is a separate and distinct entity which was considered as distinct from other forms of tuberculosis. For instance, there is Marfan's so-called "Law" which stated that individuals with healed cervical adenitis before puberty rarely contract clinically significant pulmonary tuberculosis later in life. In South Africa, it was a long-standing policy to hire native miners only if they exhibited healed scrofula, in the belief that they were then relatively immune from serious pulmonary or generalized forms of tuberculosis. This interesting policy had a certain validity as a practical measure. If a miner's scrofula was well healed, and if he did not show any other manifestations of illness, he probably had had his tuberculosis for a long time and had probably demonstrated a good capacity for self-containment of his infection. Thus, he was less likely to become ill with tuberculosis and incapacitated for mine work as a result of this disease. Unfortunately, however, he was not as certain to be free from pulmonary tuberculosis as the "Law" would dictate. Consequently, the pulmonary disease, when it existed would probably be of a chronic nature, not as likely to prevent the individual from working in the mine but quite likely to lead to the spread of tubercle bacilli to his co-workers.

Presence of other tuberculosis: The experience with the present series tends to refute the older belief that lymphatic tuberculosis is a unique entity. Almost half of the patients (58 of the 120 patients) had some form of clinically significant demonstrable tuberculosis (in addition to lymphadenitis) either before, during,

or following their original admission to the hospital. Of these 58 patients, 50 had some demonstrable pulmonary disease. Two had pleural effusions; one had an endobronchial lesion, probably secondary to lymph node erosion; 2 had genitourinary tuberculosis; 2 had bone involvement; and one had tuberculosis of the lower lip. This high incidence of clinically significant pulmonary and extrapulmonary involvement, in association with active lymph node disease, would tend to refute the earlier thinking on this subject.

Lymph node foci, just as any other pulmonary or extrapulmonary tuberculous foci, may either heal or undergo acute progression shortly following infection, or they may be quiescent for years and then demonstrate a late breakdown. It is believed very strongly that lymph node tuberculosis is not an isolated form of tuberculosis but represents one manifestation of a generalized systemic disease.

Clinical Course, Symptoms, and Signs

The presenting symptoms and signs of lymph node tuberculosis may vary widely. The patient may be seen first because of unrelated complaints, and adenopathy may be found purely in the course of routine physical examination. Most often, when the superficial nodes are involved, the patient will present himself for evaluation of a relatively asymptomatic adenopathy. The nodes may be somewhat tender and painful or they may be entirely painless. In the early stages, they are usually firm and discrete but with progression they tend to become softer, somewhat fluctuant and matted together. In the early stages, the skin overlying the lymph node is usually normal. Later on, reddening, induration, and breakdown frequently occur, sometimes with formation of chronic draining sinuses from an underlying necrotic gland.

In cases in which the mediastinal nodes are involved, the symptoms, when present, are usually secondary to lymphatic enlargement and may take the form of chronic cough, localized wheezing, or chest pain. Not infrequently, the first sign of hilar node involvement results from a secondary complication such as bronchiectasis, pulmonary hemorrhage, bronchogenic spread, or hematogenous dissemination due to rupture of a caseous node. Most frequently, however, mediastinal involvement is essentially asymptomatic and is discovered on routine roentgenographic examination. Active tuberculosis of mesenteric lymph nodes may simulate an acute abdominal process and the diagnosis will be made at exploratory laparotomy.

While most cases of lymphatic tuberculosis are relatively asymptomatic, in some instances the patient shows mild nonspecific generalized symptoms, such as malaise, gradual weight loss, and slight fever. If there is superficial node involvement or obvious mediastinal enlargement on a roentgenogram of the chest, the diagnosis is usually ultimately obtained by biopsy. If such a patient presents himself with only mesenteric node involvement, the ultimate diagnosis may be delayed until eventually localized abdominal findings result in exploratory laparotomy.

Some patients with progressive primary tuberculosis may present with deep or superficial node involvement, splenomegaly, and a septic course with fever run-

ning to 103–104° F. Most of these patients will show little, if any, pulmonary parenchymal involvement.

Another point to remember in untreated or inadequately treated lymphatic tuberculosis is the chronicity of its course with slow gradual progression and frequent exacerbations and remissions. The chronic relapsing nature of this disease has, in the past, been most discouraging for, although it was not frequently fatal, the spontaneous recovery rate was indeed small.

Diagnosis and Differential Diagnosis

The foregoing description of the disease certainly would indicate that tuberculosis must be borne in mind in the differential diagnosis of any unexplained adenopathy. Systemic infections, infectious mononucleosis, brucellosis, lymphomas, sarcoidosis, and tumors of various types might all present a similar picture. Fungal infections, which will also produce granulomatous lesions, lymphadenopathy, and calcifications on roentgenography must also be considered in the differential diagnosis.

The first point in making a proper diagnosis must, of course, be awareness. If tuberculosis is borne in mind in the differential consideration of any unexplained adenopathy, the diagnostic possibilities will not be overlooked (tables 4 and 5).

As an exclusion procedure, a tuberculin test employing a freshly prepared PPD solution is most important. A negative tuberculin reaction will virtually exclude tuberculosis, particularly if repeated four to six weeks after the onset of disease.

The positive diagnosis will usually rest on examination of material obtained by biopsy. In addition to morphologic examinations, cultures and tissue stains for acid-fast bacilli should always be done. Without bacteriologic confirmation, the diagnosis might be strongly presumed but can never be absolutely proved because other granulomas might be present. If bacteriologic confirmation can not be obtained, efforts to exclude fungal infections, such as histoplasmosis or coccidiomycosis, should be made. At times, bacteriologic confirmation may be exceedingly difficult to obtain, and treatment must be instituted on the basis of a presumed rather than a proved diagnosis. In such cases, regression of adenopathy under adequate specific chemotherapy would tend to confirm a diagnosis of tuberculosis.

TABLE 4

Criteria for Diagnosis

Clinical plus positive tuberculin reaction	6
Positive bacteriologic findings on pus from lymph node	5
Morphology of biopsy material without bacteriologic proof	41
Morphology of biopsy material plus positive bacteriologic findings	57
Proved tuberculosis elsewhere plus adenopathy	11
	120

TABLE 5

PATIENTS WITH TUBERCULOSIS IN ADDITION TO LYMPHADENITIS ON ORIGINAL FITZSIMONS ARMY HOSPITAL ADMISSION RELATED TO STATUS OF LYMPH NODES ON ADMISSION AND OTHER TUBERCULOSIS PRIOR AND SUBSEQUENT

Type Nonlymphatic TB on Admission	Number	Lymph Nodes	Number	Prior TB Other than Lymphatic	Number	TB Developing During or Following Original Admission	Relapses		
							Prior to Therapy		Subsequent to Therapy
Pulmonary, minimal, inactive	12	Cervical	9	Pulmonary lesion:		0	Pulmonary	0	0
		Axillary and cervical	1	Known	6		Nodular	6	1
		Mediastinal	2	Unknown	6		Both	0	0
Pulmonary, minimal, active	21	Cervical	10	Pulmonary lesion:		TB peritonitis one	Pulmonary	0	0
		Mediastinal	4	Known	1	month after ad-	Nodular	2	1
		Cervical and mediastinal	4	Unknown	18	mission (led to mesenteric nodes)	Both	0	0
		Axillary	2	Pre-treatment on old film	1	-1			
		Mesenteric	1	Pleural effusion	1				
Pulmonary, moderately advanced, active	9	Cervical	4	Pulmonary lesion:			Pulmonary	0	0
		Inguinal and mediastinal	1	Known 3 years as minimal			Nodular	0	1
		Axillary	1	No therapy	1	0	Both	0	1
		Axillary and mediastinal	1	Unknown	8				
		Cervical and mediastinal	1						
		Mediastinal	1						
Pulmonary, moderately advanced, active undetermined	1	Cervical	1	Pulmonary lesion:		?			
				Unknown	1	AWOL		0	? AWOL
Pulmonary, far advanced, active	1	Cervical	1	Pulmonary lesion:					
				Unknown	1	0		0	? AMA
Pulmonary, minimal, active and pleural effusion	1	Cervical	1	Pulmonary lesion:					
				Unknown	1	0		0	0

Pulmonary, minimal, emphysema	1	Mediastinal and cervical	1	Pulmonary lesion: Unknown	1	0		0	0
Minimal, active and endobronchial	1	Mediastinal	1	Pulmonary lesion: Unknown	1	0		0	0
Endobronchial	1	Mediastinal	1	Pulmonary lesion: Unknown	1	0		0	0
Pulmonary, far advanced, active and pleural effusion	1	Mediastinal	1	Pulmonary lesion: minimal on old film reviewed	1	0		0	0
Pleural effusion	2	Inguinal	1	Pulmonary lesion:					
		Mediastinal	1	Unknown	2	0		0	0
Lower lip	1	Mediastinal	1	Unknown	1	0		0	0
Bone (clavicle) and pericarditis	1	Mediastinal	1	Unknown	1	0		0	0
Bone	1	Cervical	1	Unknown	1	0	Nodes	1	0
Genitourinary	2	Mediastinal	1	Unknown	2	0		0	0
		Axillary	1						
None on admission, but non-nodular TB before original admission	1			Pulmonary lesion: Bone			Node	1	0
		Cervical	1	TB and pleural effusion	1	0	Pleural effusion and bone		
None on admission, but non-nodular TB following admission	1	Cervical and axillary	1		0	Pulmonary, moderately advanced: 6 weeks following admission-1	0		Node-1

Treatment

In the past, there was a great deal of controversy and confusion regarding the treatment of lymphatic tuberculosis. There were those who considered this an essentially benign disease requiring little or no treatment. This was especially true of those writers whose statistics were heavily loaded with data on children in the five to twelve age group. Others, considering lymphatic tuberculosis as an essentially separate form of the disease, tended to separate the treatment of lymphatic tuberculosis from the therapy of other forms. From the preceding sections of this paper, it is obvious that the present writers consider this concept to be fallacious.

Most of the earlier writers believed that lymphatic tuberculosis was a chronic relapsing and progressive disease which caused disability and, therefore, should be treated. However, there was certainly no agreement on what constituted adequate therapy. During the pre-chemotherapy era, most patients were treated with either one or a combination of the following modalities: rest, X-ray or ultraviolet irradiation, aspiration and drainage, tuberculin therapy, or surgical resection. The surgical resection might range anywhere from mere local excision to a radical cancer type of gland dissection. These and many other ideas and methods, including the "Royal Touch," were tried and seem to have had only one thing in common, namely, uniformly variable results with generally poor outlook for eventual permanent recovery. Because of the chronically relapsing nature of this disease, the results as reported by the earlier writers were most confusing.

With the advent of modern drug therapy and the realization that lymphatic tuberculosis is but one facet of a generalized systemic disease, treatment has become more rational and results have become more predictable. In the present experience, three factors seem to be of major importance in the chemotherapy of tuberculosis in general, including lymph node tuberculosis in particular, namely, the choice of a drug regimen, the dosage of the drug used, and the duration of treatment (tables 6 and 7).

In 19 of the patients in the present series, the original Fitzsimons Army Hospital admission represented a relapse of previously known lymphatic tuberculosis. Ten additional patients experienced relapse of lymph node disease subsequent to their first admission to this hospital. In these 29 patients, a study of the unsuccessful treatment regimens used serves to emphasize some significant and interesting facts regarding what constitutes proper therapy. Ten of these 29 patients (table 8) had no chemotherapy during their original episode of tuberculous lymphadenitis. In all of these patients, the original episode of lymph node disease predated the introduction of chemotherapy. One patient had some form of short-term chemotherapy, but the details of the regimen are unknown.

Eight patients had received their initial chemotherapy in the early days of drug treatment and received streptomycin alone. Of these 8 patients, 6 received short courses of streptomycin with immediate satisfactory results, only to suffer relapses. One patient had a relapse following six months of treatment with

TABLE 6

TREATMENT REGIMENS: INITIAL FITZSIMONS ARMY HOSPITAL TREATMENT

VI *A*		VI *B*		VI *C*	
Drugs Used	Patients	Duration of Chemotherapy	Patients	Surgery	Patients
None	3	None	3	None	21
Streptomycin alone (all before 1950)	5	Less than 90 days	18*	Diagnostic biopsy; peripheral node	84
Streptomycin-PAS	43	3–6 months	42*	Thoracotomy with biopsy or resection of hilar nodes	6
Streptomycin-Isoniazid	40	6+–12 months	30*		
Streptomycin-PAS-Isoniazid (either triple or in changing combinations)	27	12+–18 months	26	Pulmonary resection	5
		More than 18 months	1	Resection left clavicle—Bone TB	1
Streptomycin-viomycin followed by streptomycin-PAS	1			Exploratory laparotomy-mesenteric nodes and TB peritonitis	1
Streptomycin-oxytetracycline	1			Radical node dissection	1
				Bronchoscopy with biopsy	1
	120		120		120

* Includes patients transferred to Veterans Administration Hospital with no follow-up information but who presumably had treatment continued after transfer. Also includes patients discharged with advice to continue drugs longer but in whom there was no follow-up confirmation that drugs were actually continued.

streptomycin alone, and one experienced a relapse after a twelve-month course had been completed. Thus, 8 of the 10 patients who received streptomycin alone suffered subsequent relapses of their disease.

Nine patients received a streptomycin-PAS regimen as the initial treatment, with immediate good results and later relapse. In addition to this, 4 patients whose previously treated disease had relapsed were given streptomycin-PAS only to develop an additional recurrence at a later date. It is noted, however, that, with one exception, all of the unsuccessful streptomycin-PAS regimens consisted of less than five months of total therapy. The one exception was a patient who had had streptomycin-PAS for three years and was believed to represent an example of continued activity of widespread extrapulmonary tuberculosis rather than a true relapse. This man's disease was subsequently treated to the stage of inactivity by the use of streptomycin and isoniazid. Undoubtedly, in this case streptomycin-PAS was biologically inadequate therapy and the administration of isoniazid in effective dosage was needed to rectify the situation.

In the present series, only one patient who received isoniazid had a subsequent relapse. This man had had isoniazid-streptomycin-PAS therapy for only three months for active cervical and axillary adenopathy and a freshly developing, moderately advanced pulmonary disease, at which point he left the hospital

TABLE 7

CHEMOTHERAPY IN 19 PATIENTS WITH TUBERCULOSIS ADMINISTERED PRIOR TO ORIGINAL FITZSIMONS ARMY HOSPITALIZATION

(In these patients original Fitzsimons Army Hospital admission represented a relapse of old lymphatic tuberculosis)

Drug	Number	Duration
None	8	8—None
Streptomycin	5	1–2 weeks
		1–2 months
		1–5 months—3 courses
		1–6 months
		1–12 months
Streptomycin-PAS	5	1–3 months
		2–4 months
		1–5 months—2 courses
		1–3 years Fitzsimons Army Hospital, admission believed to be residual of widespread extrapulmonary TB rather than relapse
Unknown regimen	1	1–3 months
Total	19	19

without permission. One year later he was readmitted for a relapse of active cervical adenitis. However, his axillary lymph node disease and his pulmonary lesion were considered to be inactive at the time of his readmission. His disease responded well to re-treatment, which consisted of isoniazid-streptomycin for eleven months, followed by isoniazid for thirteen months. He is currently well with no evidence of tuberculous activity.

It might be mentioned that occasionally in the present series an infected node has been seen to enlarge shortly after the institution of isoniazid therapy. Some nodes which were originally rather large have been observed to become fluctuant, even necessitating drainage shortly after the institution of isoniazid in adequate dosage. All of these cases, however, subsequently have shown good regression and healing with the continuation of the drug. One might postulate that this represents an acute reaction subsequent to the sudden release of large amounts of cellular debris and nuclear material as a consequence of rapid destruction of large numbers of tubercle bacilli in the node. It is to be noted that this occasional reaction has been observed only in the isoniazid-treated cases, probably because of the greater potency of this agent.

It might also be mentioned that mediastinal lymph nodes, which may represent an active primary reaction, will sometimes persist or even enlarge temporarily under chemotherapy. This has also been the experience with mediastinal nodes on the Pediatric Service where it has been noted that chemotherapy does not particularly influence the course of the mediastinal lymph node enlargement.

These observations regarding immediate progression of nodes shortly after the onset of isoniazid therapy are in no way original in this series of cases. This phenomenon has been recognized, and reported on several occasions since the advent of isoniazid therapy.

In general, patients on an adequate drug regimen show a rather prompt recession of the more acutely involved lymph node groups. In many cases, hard shotty residual adenopathy remains unchanged indefinitely following completion of treatment. In other cases, especially if the disease is treated before great permanent anatomic change arises in the node, the involved lymph node groups will "melt" away and disappear entirely. Any generalized systemic manifestations tend to disappear rather promptly in all cases.

A few words should be said concerning the current status of surgery in lymphatic tuberculosis. The writers believe that there is virtually no indication for radical lymph node dissection for tuberculous lymphadenitis. In the present series, surgery has been limited mainly to diagnostic biopsy. Surgery is also indicated for removal of grossly fluctuant nodes in which rupture is believed to be imminent, with an attendant risk of massive spread of the infection. Moreover, surgery is also indicated for the excision of significant secondary residuals after the completion of chemotherapy. For instance, persistently atelectatic or bronchiectatic portions of a lung resulting from long-standing extrinsic bronchial obstruction should be removed if productive of significant symptoms or if they are the basis of significant complications.

On the basis of the present experiences, including analysis of the therapeutic failures in 29 patients with relapsing disease, it is believed that all patients should receive multiple-drug therapy and that isoniazid should be one of the drugs used. It is also believed that it is important to continue chemotherapy at least to the stage of disease inactivity in accordance with National Tuberculosis Association standards, i.e., until six months after all signs of activity have disappeared.

Currently, the patients with lymphatic tuberculosis are receiving isoniazid-PAS or isoniazid-streptomycin for an average duration of eighteen to twenty-four months. The dose of isoniazid employed has been at least 300 mg. per day in all cases. More recently, there has been a tendency to increase the isoniazid dosage above this level in many instances and to attempt to adjust the dose to the concentration of drug determined to be present in the blood. The experience in this hospital to date does not indicate any significant differences between isoniazid-PAS and isoniazid-streptomycin in the present series.

Results of Follow-Up Observations (Table 9)

As the result of the foregoing principles of therapy, it is believed that the over-all results have been quite satisfactory. Of the 120 patients, 50 have been followed for longer than two years. Of these, 48 are apparently well and working, and 2 have recently been lost to follow-up study. None are known to be ill, at the present time.

Seventy of the patients have been followed for less than two years. Of these, 36 were still considered as having active disease at the time they were transferred to veterans hospitals, and no current information is available as to their status. There were 18 patients whose tuberculosis was considered inactive at the time of their discharge but for whom no current information is available. In the remaining 16 of these 70 patients followed for less than two years, current

TABLE 8

RELAPSE SUBSEQUENT TO FIRST FITZSIMONS ARMY HOSPITAL ADMISSION (TOTAL PATIENTS—10)

First Hospital Admission					Relapse			
Race	Date	Disease	Drug	Durations	Date	Disease	Drug	Duration
				months				*months*
N	4/1949	Anterior cervical—left, pulmonary, moderately advanced, active	Dihydrostreptomycin	4	5/53	Anterior cervical-right, pulmonary, minimal, active	Streptomycin-isoniazid	7+
N	3/1952	Cervical—right	Streptomycin-PAS	4	1/55	Cervical—r ght	Streptomycin-isoniazid	12+
N	7/1951	Cervical—bilateral	Streptomycin-PAS	2	1/53	Cervical—bilateral	Streptomycin-isoniazid, PAS	16
O	4/1950	Cervical—right, mediastinal	Streptomycin-PAS	4	6/52	Cervical—right	Streptomycin-PAS	6
N	6/1950	Cervical—right, tonsil	Streptomycin	1	11/51	Anterior cervical	Streptomycin-PAS	½ AWOL
W	5/1950	Cervical and pulmonary, minimal	Streptomycin-PAS	4½	12/51	Cervical	Streptomycin-PAS, viomycin	11
W	2/1948	Pulmonary, moderately advanced, active	Streptomycin	5	8/50	Axillary—right	Streptomycin-PAS	1
			(3 courses)		8/51	Axillary—right	Streptomycin-PAS	Less than 1
			Right upper lobectomy and right thoracoplasty		6/52	Axillary—right	Streptomycin-PAS	5
							Streptomycin-isoniazid	3

N	6/1945	Cervical—right	0	0	5/47	Cervical—right	0	0
					7/48	Pleural effusion—left	0	0
					2/52	Cervical—bilateral	Streptomycin-PAS	4
					4/53	Cervical—right	Streptomycin-isoniazid	8*
N	5/1953	Anterior cervical—left; axillary—left; mediastinum—left (pulmonary, moderately advanced, active, both upper lobes, developed 7/1953, six weeks after admission)	Streptomycin-isoniazid, PAS	3 AWOL	6/54	Cervical—right (no relapse of axillary or mediastinal nodes; no active pulmonary lesion)	Streptomycin-isoniazid (isoniazid-PAS)	11 at maximal hospital benefit (13 outpatients after inactivity)
Spanish-American	7/1949	Mediastinal	0	0	1949–1954	Many short admissions for cervical, axillary, or mediastinal	0	Never stayed long enough
					3/54	Generalized	Streptomycin-isoniazid	2 weeks — AWOL unimproved

TABLE 9

FOLLOW-UP OBSERVATIONS FROM ORIGINAL ADMISSION

0–6 months	28
6–12 months	14
1–2 years	28
2–3 years	21
3–4 years	21
4–5 years	6
5+ years	2
	120
50—Followed 2 years or more:	
Status unknown	2
* PR for tuberculosis	0
Apparently well and working	48
	50
70—Followed less than two years:	
Status unknown (active on transfer to VA hospital)	36
No information since discharge, inactive at discharge and presumably well	18
Apparently well and working on follow-up	16
* PR for tuberculosis	0
	70

* PR—Permanently retired from military service.

information is available to the effect that they are all apparently well and working.

Thus, of the 120 patients, with elimination of the 36 patients who were transferred to the Veterans Administration hospitals while still under active treatment, and whose disease was considered active at the time of their removal from Fitzsimons Army Hospital, there are no known currently active cases or reactivations in the series treated or re-treated by adequate modern chemotherapy.

CASE REPORTS

The following 3 cases are presented to illustrate some of the problems described in the foregoing sections of this paper:

Case 1. The patient, a 25-year old Negro male, was originally admitted to the hospital on June, 1945, with right cervical adenitis. The diagnosis was established by microscopy of pus from a draining lymph node. Treatment consisted of bed rest for five months, with good clinical clearing of his adenopathy.

In May, 1947, the patient had recurrence of his right cervical adenitis. No chemotherapy was given. He received bed rest for five months, ultraviolet radiation, and aspirations of the infected nodes. His condition improved and he was discharged in November, 1947, again with complete clearing of the lymphadenitis.

In July, 1948, the patient developed a left pleural effusion for which he received penicillin for three weeks, without result. He was treated with bed rest, being discharged in June, 1949. There was no recurrence of adenopathy at this time. The effusion was considered to be of tuberculous etiology.

In February, 1952, the patient was again admitted with bilateral anterior and posterior cervical adenitis. This time, he had a short course (four months) of streptomycin-PAS. He apparently responded well and was discharged in August, 1952. In August, 1953, he again had a reactivation of right cervical adenitis. This time he had isoniazid-streptomycin in the hospital for eight months and was discharged in June, 1954, to continue drugs for twelve months more as an outpatient. He is now well and working.

Comment: This case is an excellent example of the chronic relapsing nature of untreated or inadequately treated lymphatic tuberculosis. The tendency to spontaneous remission and exacerbation was quite common in the earlier days and probably contributed a good deal to the confusion regarding the effectiveness of various therapeutic measures. It may also be noted that the patient experienced a relapse of his disease after a relatively short course of chemotherapy, to which he had shown an initial excellent response. There has been no relapse following re-treatment with a more intensive drug regimen.

Case 2. A 24-year-old Negro male was admitted to Fitzsimons Army Hospital in August, 1950, for bilateral hilar adenopathy. The diagnosis was proved by examination of biopsy material obtained at an exploratory thoracotomy. The patient was treated with bed rest and received streptomycin-PAS for only four months. His hilar lymph nodes showed marked regression and he was discharged in February, 1952. He was subsequently returned to active duty in 1954. He remains well.

Comment: This case shows the marked regression that may occasionally occur even in the mediastinal nodes. This patient certainly received an inadequate period of chemotherapy by today's standards. Nevertheless, he has apparently made an eminently satisfactory recovery.

Case 3. A 25-year-old white male was admitted to the hospital in December, 1952. Because of huge mediastinal nodes, he was originally considered to have a lymphoma. Exploratory thoracotomy showed extensive active tuberculous mediastinal lymphadenitis. A review of his chest film taken on entrance to military service in 1948 showed mediastinal nodes, definitely enlarged but smaller in size. The patient was given chemotherapy for twelve months without any marked change in the roentgenographic appearance of the nodes, and he was eventually discharged as having obtained maximal hospital benefit in April, 1954. No current chest film is available, but in April 1956, the patient wrote that he was well and without symptoms and working a full eight-hour day.

Comment: This case illustrates the occasionally confusing diagnostic possibilities of lymphatic tuberculosis. Clinically, this man seemed to have a lymphoma which turned out to be massive mediastinal lymph node tuberculosis. An exploratory thoracotomy confirmed the diagnosis and resulted in institution of the proper therapy. The patient's experience certainly presents a good argument for diagnostic thoracotomy when indicated rather than empiric so-called "therapeutic trials". While the clinical response in this patient was excellent, no dramatic changes were noted on roentgenography even though he received adequate therapy. This is an example of mediastinal node disease in which the lymph nodes fail to show substantial shrinkage even after adequate therapy

and even though the patient remains well clinically. This case represents the other extreme in the scale from the second case in which the lymph nodes virtually disappeared after somewhat inadequate therapy.

Summary

Of 3,958 cases of active tuberculosis observed on the Pulmonary Disease Service of Fitzsimons Army Hospital between January 1, 1952, and December 31, 1955, there were 120 cases of active lymph node tuberculosis. Patients from the Pediatric Service (less than fourteen years of age) have been excluded from consideration.

On the basis of the experience with patients with lymph node tuberculosis it is believed that the following conclusions are justified:

Lymphatic tuberculosis is just one facet of a generalized systemic disease and should be treated as such. It is not a separate or distinct entity.

A relatively high incidence of mediastinal lymph node involvement and a very low incidence of mesenteric nodal involvement were noted in this series. This seems to indicate the increasing importance of primary pulmonary tuberculosis in the adult and the decreasing significance of the gastrointestinal tract as a portal of entry for primary infection, as well as the declining importance of gastrointestinal tuberculosis in general.

All cases of lymphatic tuberculosis should receive adequate chemotherapy. Multiple-drug regimens, utilizing isoniazid as one of the drugs, should be employed in all cases and continued at least to the point of apparent inactivity of the disease. Currently, treatment for eighteen to twenty-four months would be the procedure of choice. There seems to be no great difference between isoniazid-PAS or isoniazid-streptomycin regimens in the results obtained.

Except for diagnostic biopsy, which should be done in all cases (if possible), surgery plays a relatively minor role in the modern management of lymphatic tuberculosis. It continues to be indicated for removal of fluctuant nodes with imminent danger of rupture and for the correction of significant secondary residuals, such as symptomatic atelectatic or bronchiectatic lung areas after adequate chemotherapy has been given.

The prognosis of adequately treated lymph node tuberculosis today appears to be excellent, with a very low relapse rate and an excellent outlook with respect to return to a normal useful existence within a relatively short period of time.

Sumario

El Estado Actual del Tratamiento de la Tuberculosis Linfática

Entre 3,958 casos de tuberculosis activa, observados en el Servicio de Enfermedades Pulmonares del Hosipital Militar Fitzsimons entre el 1° de enero de 1952 y el 31 de diciembre de 1955, hubo 120 casos de tuberculosis activa de los ganglios linfáticos. No se han tomado en cuenta los enfermos del Servicio de Pediatría (de menos de catorce años de edad).

A base de lo observado en los enfermos con tuberculosis de los ganglios linfáticos, parece que las siguientes conclusiones están justificadas:

Notáronse en esta serie una incidencia relativamente alta de invasión de los ganglios mediastínicos y una incidencia muy baja de invasión de los ganglios mesentéricos. Esto parece indicar la creciente importancia que reviste la tuberculosis primaria en el adulto y la decreciente importancia del tubo gastrointestinal como vía de entrada para la infección primaria, así como la menguante importancia de la tuberculosis gastrointestinal en general.

Todos los casos de tuberculosis linfática deben recibir quimioterapia adecuada. En todos, deben emplearse regímenes polimedicamentosos, utilizando la isoniacida como una de las drogas, y continuándolos a lo menos hasta observar aparente inactividad de la dolencia. Actualmente, el procedimiento de elección sería de dieciocho a veinticuatro meses de tratamiento. En los resultados obtenidos, no parece que haya mucha diferencia entre los regímenes de isoniacida-PAS y de isoniacida-estreptomicina.

Aparte de la biopsia diagnóstica, que debe ejectuarse (si es posible) en todos los casos, la cirugía desempeña un papel relativamente secundario en la asistencia moderna de la tuberculosis linfática. Sigue estando indicada para la erradicación de los ganglios fluctuantes en riesgo inminente de rotura y para la rectificación de los residuos secundarios de importancia, tales como zonas pulmonares atelectáticas o bronquiectáticas sintomáticas que subsisten después de administrar quimioterapia adecuada.

El pronóstico de la tuberculosis de los ganglios linfáticos, tratada adecuadamente, parece ser excelente hoy día, con una tasa muy baja de recidivas y una magnífica perspectiva en cuanto al retorno a una existencia útil y normal en un período de tiempo relativamente breve.

Resume

Etat actuel et traitement de la tuberculose lymphatique

Sur 3.958 cas de tuberculose en activité observés dans le service des maladies pulmonaires de Fitzsimons Army Hospital entre le 1er janvier 1952 et le 31 décembre 1955 il y a eu 120 cas d'adénopathies tuberculeuses en activité. Les malades du service de pédiatrie (enfants au-dessous de 14 ans) ont été exclus de l'analyse.

Sur la base de l'expérience acquise parmi les malades atteints de tuberculose ganglionnaire, les auterus considèrent que les conclusions suivantes sont justifiées:

La tuberculose lymphatique n'est qu'un des aspects de la maladie tuberculeuse généralisée et doit être traitée comme telle. Ce n'est pas une entité séparée ou distincte.

Une fréquence relativement élevée de l'atteinte des ganglions médiastinaux et une très faible fréquence de l'atteinte des ganglions mésentériques ont été observées dans cette série. Ceci semble indiquer l'importance croissante de la primo-infection tuberculeuse chez l'adulte et la réduction de la tuberculose gastro-intestinale en général.

Tous les cas de tuberculose lymphatique doivent être soumis à une chimiothérapie adéquate. Les associations médicamenteuses utilisant l'isoniazide comme l'une des drogues doivent être employées dans tous les cas et le traitement poursuivi, au minimum, jusqu'à stabilisation de la maladie. Actuellement, la cure de dix-huit à vingt-quatre mois serait la thérapeutique de choix.

Il ne semble pas qu'il y ait une grande différence entre le traitement isoniazide-PAS et isoniazide-streptomycine dans les résultats obtenus.

Sauf pour la biopsie diagnostique, qui devrait être effectuée dans tous les cas (si possible), la chirurgie ne joue qu'un rôle relativement minime dans la thérapeutique moderne dirigée contre la tuberculose lymphatique. La chirurgie continue à être indiquée pour la résection des ganglions fluctuants menacés de rupture et pour la correction de séquelles secondaires importantes, telles que segments pulmonaires affectés d'atélectaise symptomatique ou de bronchiectasie après qu'une chimiothérapie adéquate ait été faite.

Le pronostic de la tuberculose des ganglions lymphatiques traitée de façon adéquate paraît désormais excellent, le taux de rechûte est très bas et la perspective est excellente en ce qui concerne le retour à une existence normale et active dans un délai relativement court.

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TUBERCULOUS MENINGITIS[1, 2]

A Report on Fifty-Four Consecutive Cases of Children Treated with Antimicrobial Drugs and Purified Protein Derivative: Bacteriology, Pathology, Electroencephalographic and Psychometric Follow-up Observations

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Introduction

This report presents the results of treatment of 54 consecutive cases of tuberculous meningitis in children admitted to the Alexandra Hospital between August, 1950, and September, 1955. Successful therapy in tuberculous meningitis began less than ten years ago and the history in general is well known. Recently a lucid and concise review of the therapy of this disease was presented by Weinstein and Meade (1). In brief, reports of the first five years of treatment of tuberculous meningitis were concerned chiefly with streptomycin therapy, with emphasis on survival, while later reports have dealt mainly with streptomycin in association with other drugs, with emphasis on the completeness of recovery. It is evident from reports at the present time that a substantial survival rate is to be expected from the treatment of tuberculous meningitis; yet the actual recovery rate from this disease remains somewhat obscure, due to the lack of adequate follow-up reports. In particular there are few pertinent data in regard to controlled psychometric evaluations of electroencephalographic findings in patients who have recovered from tuberculous meningitis, indicating the degree of intellectual sequelae suffered by these patients.

The scanty literature on the use of purified protein derivative (PPD) in the treatment of tuberculous meningitis is evidence that this substance has received relatively little clinical use since the time of its introduction by Smith (2) in 1950. At the time of the introduction of PPD as an adjunct to therapy in tuberculous meningitis, the survival rate for that disease in Montreal was deplorably low. The rationale given for treatment with PPD by Smith and Vollum was presented in so logical a fashion that it was decided to incorporate this substance in the therapy of *all* patients admitted to the Alexandra Hospital for tuberculous meningitis for an indefinite period of time. Encouraged by a striking initial success, it was considered to be advantageous to continue with the use of PPD in combination with other drugs, and the observations made over the past five years are here presented.

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Methods and Materials

Treatment

Three principles were recognized after the experience of previous failures at therapy, viz.:

1. That the finding of a cerebrospinal fluid that was "almost normal" still indicated active disease, regardless of the absence of abnormal clinical signs in the patient. Heretofore, an attempt was made to stop therapy when the protein, sugar, and chloride values of the cerebrospinal fluid were "almost normal," and invariably failure of therapy was evident when a recurrence of the clinical disease ensued. Therefore, it was planned to continue therapy until such time as the findings in the cerebrospinal fluid became normal and remained normal for a period of several months.

2. That the duration of therapy obviously must be prolonged, certainly beyond three months, the time during which it had previously been given, and until the cerebrospinal fluid findings returned to normal.

3. That the exudate, partially or completely blocking the basal cisterns of the brain, must be removed and, if possible, its formation prevented. It was recognized that this tuberculous exudate at the base of the brain, preventing the free flow of

TABLE 1

Drugs and Dosage Employed

Drug	Dosage
Intramuscular streptomycin	1.0 gm. a day given initially and daily for 200 successive days thereafter; reduced during second year to 0.5 gm. daily for children less than 6 years old.
Intrathecal streptomycin	Given daily for first 40 days; a "rest period" for 7 days; thereafter given every second day for 40 doses with a second "rest period" of 7 days, and finally 40 doses until the end of therapy. Reduced from 50 to 25 mg. during second year; given daily for first 40 days only during fifth year.
PPD*	Initial dose of 0.0000025 mg. given on eighth day; if reaction is observed, e.g., fever, headache, rise in pulse, vomiting, pleocytoses in cerebrospinal fluid, the same dose is repeated 4 days later; if no reaction is observed, then a double dose is given 2 days later until a reaction is observed, whereupon this same dose is repeated 4 days later until no reaction is again observed.
PAS	Given on the basis of body surface area, 10 gm. per square meter of body surface; frequently vomited during early weeks of illness and amounts retained were often questionable; discontinued during most of fourth and all of fifth year.
Isoniazid	First used during third year in 3 patients. Given on basis of body weight, 10 mg. per kg. each day for first 2 weeks; thereafter 5 mg. per kg. daily.

* Manufactured by Parke, Davis & Co.

TABLE 2

CHANGES IN THERAPY IN RELATION TO SURVIVAL AND SEQUELAE

	1948–1950	First Year	Second Year	Third Year	Fourth Year	Fifth Year
Duration of therapy	2–3 months	200 days	200 days	200 days	200 days	200 days
Intramuscular streptomycin	1 gm. daily	1 gm. daily	0.5–1 gm.	0.5–1 gm.	0.5–1 gm.	0.5–1 gm.
Intrathecal streptomycin	50–100 mg.	50 mg.	25 mg.	25 mg.	25 mg.	25 mg. (first 40 days)
PPD	No	Yes	Yes	Yes	Yes	Yes
PAS	No	Yes ±	Yes ±	Yes ±	Yes ±	No
Isoniazid	No	No	No	Yes ±	Yes	Yes
Adequate control of intracranial pressure	No	No	No	No	Yes	Yes
Early mobilization	No	Yes	Yes	Yes	Yes	Yes
Physiotherapy	No	No	No	Yes	Yes	Yes
Nutritional care	Adequate	Good	Good	Good	Good	Good
Number of patients	10	11 (1 hopeless)	10 (2 hopeless)	15 (1 hopeless)	13	5
Survival	None	9	6	11	13	5
Sequelae:	None					
Deaf	—	7	—	—	—	—
Orthopedic	—	2	2	—	—	—
Severe loss of brain capacity	—	—	—	3	1	1
Recovery	None	2	4	8	12	4

the cerebrospinal fluid, and its eventual absorption were chief contributing factors to brain damage and eventual death.

To this end a plan of therapy was adopted by the Tuberculosis Unit at the Alexandra Hospital incorporating these principles. This plan has undergone a number of modifications for reasons that will be evident, but essentially the original plan has been preserved.

The drugs employed and the doses given at the beginning of this work are set forth in table 1. In brief there were three "courses" of therapy with a "rest period" of one week between each course, the duration of courses having been determined by the favorable outcome of the first patients treated.

The initial course of therapy for the first patients was for forty days, streptomycin being given intrathecally (50 mg.) and intramuscularly (1.0 gm.), PAS orally each day, and purified protein derivative of *M. tuberculosis* (PPD) on every second or fourth day, depending on whether a clinical response to this substance was observed. After a "rest period" of one week, a second course was given for eighty days. During this second course intrathecal streptomycin (50 mg.) was given on alternate days, intramuscular streptomycin (1.0 gm.) daily and PPD again, depending on the frequency of a clinical reaction. On the completion of the second course, a second "rest period" of one week was observed,

after which time a third course similar to the second was given. The "rest periods" were introduced originally as a necessary reprieve from injections. Since there was no apparent contraindication for these "rest periods", they have been maintained for the psychologic benefit of the children.

In table 2 are illustrated in a comprehensive manner the therapy and its changes, with corresponding elimination of sequelae and reduction of mortality, in an attempt to present those factors which are considered to have contributed most to recovery.

During the period from 1948 to August, 1950, no child survived tuberculous meningitis, although a longer survival period after diagnosis was observed with apparent but only temporary arrest of the disease. On the other hand, a similar number of patients were treated during August, 1950, through July, 1951, and 9 of 11 patients not only survived but apparently recovered from the disease. It is noteworthy that three changes in therapy were made during that year, viz., prolongation of therapy from about three months to seven months; administration of PPD intrathecally, as was recommended by Smith; and the use of PAS orally (frequently causing vomiting).

Observations

Having witnessed this unprecedented recovery of 9 of 11 patients, 2 having died chiefly from other causes, it was only logical to pursue further the use of PPD, for at the time it appeared that the survival rate was considerably higher than was to be expected either from prolongation of the period of therapy or the addition of PAS. Subsequent reports have borne out this impression, for they indicate that the over-all survival rate has been less when a similar plan of therapy was employed without PPD (table 3).

TABLE 3*

Para-Aminosalicylic Acid and Streptomycin Treatment of Tuberculous Meningitis

Investigator	Drug Used and Route	Results of Treatment		
		Number of Patients	Number Surviving	Per Cent Surviving
Des Autels (3)	Streptomycin, intramuscularly; PAS, per os	15	9	60.0
Lorber (4)	Streptomycin, intramuscularly, intrathecally; PAS, per os	38	28	73.7
Spies (5)	Streptomycin, intramuscularly, intrathecally	21	4	19.0
Fitzpatrick (6)	Streptomycin, intramuscularly; PAS, per os	22	9	40.9
Public Health Service (7)	Streptomycin, intramuscularly, intrathecally	47	21	44.7
Totals		143	71	49.4

* After Weinstein and Meade (1).

Unfortunately, 7 of these 9 children who survived were deaf and had complete loss of vestibular function. There was no apparent intellectual impairment in this group of children and the problem of immediate clinical concern was the elimination of gross clinical deafness. It was reasoned that excessive amounts of streptomycin (50 mg.) were being introduced intrathecally, in far greater concentrations than were necessary to be inhibitory for any *M. tuberculosis* liberated into the cerebrospinal fluid; likewise it was reasoned that 1.0 gm. of streptomycin given intramuscularly each day was excessive, certainly for an infant or a small child. Thereafter an arbitrary dose of 0.5 gm. was given to all children less than six years of age, and streptomycin (25 mg.) was given intrathecally to all except 4 infants less than two years of age, each of whom received 15 mg. of streptomycin intrathecally. During that year and subsequently no patient with gross clinical deafness has been observed, and the elimination of this serious complication has been related to the reduced dosage of intrathecal streptomycin. (Careful otologic studies have been made in all patients described in this report and they will be presented in a separate report by Dr. H. E. McHugh.)

In July, 1952, it was apparent that an unprecedentedly high recovery rate could be expected in tuberculous meningitis; gross clinical deafness apparently could be eliminated, and up to that time no instance of serious intellectual

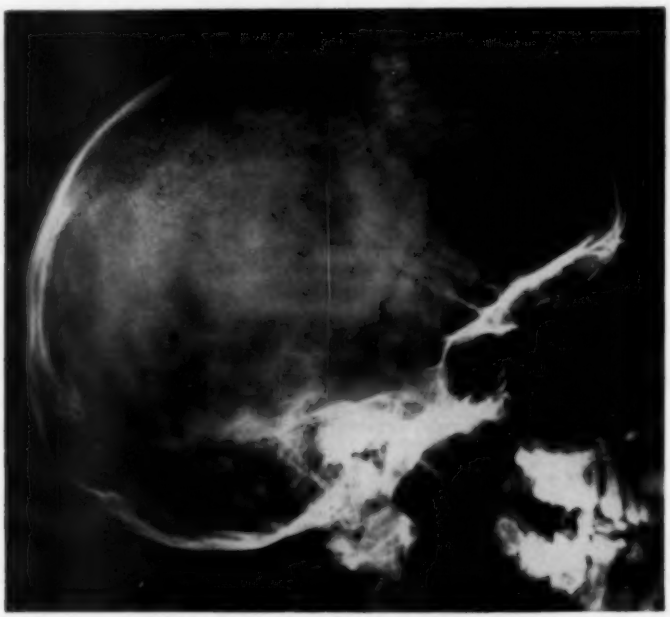

FIG. 1. Illustration showing suprasellar calcification in a case of arrested tuberculous meningitis five years after completion of therapy. Calcification was first evident three years after the discontinuance of therapy and similar areas of calcification have been demonstrated in 10 other children.

sequelae had been encountered. It also appeared that several instances of orthopedic deformities that had occurred could have been prevented and that such sequelae could be minimized or eliminated by adequate physiotherapy during the period of acute illness.

During the following year, 15 patients were treated and for the first time serious intellectual sequelae were encountered in 3 patients and, contrary to expectations, four deaths occurred. It was at this time that there was some disagreement locally as to the necessity for intracranial "burr holes" and intrathecal streptomycin therapy. For a while it was evident that a number of the patients were doing poorly because of failure to control intracranial pressure in this particular group. Furthermore, it was evident that one certain favorable feature of the intrathecal administration of streptomycin was that it afforded an opportunity to relieve increased intracranial pressure. During this most difficult period it became obvious that careful control of intracranial pressure was indeed a vital procedure in the treatment of tuberculous meningitis, and to this end our efforts were directed. One patient (*JB-28*) represents the turning point of therapy in this respect, and reference to this patient will be made subsequently.

It is agreed that the results during the following year are open to a degree of speculation, for not only was this the first year when adequate control of intracranial pressure had been effected, but the present group is also the first entire group to whom isoniazid was given, although it had been used on 3 patients the previous year. One does not wish to depreciate the value of isoniazid in the therapy of these children, yet it is only fair to point out that it was the opinion of the present writers that control of intracranial pressure was responsible to a significant degree for the favorable results observed.

During the past year 5 patients have been treated, and for the first time intrathecal streptomycin (25 mg.) was used during the first forty days of therapy only, for bacteriologic studies indicated that in only a few patients was viable *M. tuberculosis* recovered after the first forty days of therapy. The fifth and last patient treated by us during the past year died before treatment was completed, representing the first death of a patient admitted to the Unit since 1953. It is of particular significance that this patient was treated with high therapeutic doses of isoniazid, PAS, streptomycin intramuscularly, and cortisone over a period of three months, with a course from a relatively good clinical condition to one of progressive deterioration. As a last resort PPD was added to therapy, without apparent clinical benefit.

The length of this communication does not permit detailed remarks in regard to the many interesting observations made during the treatment of these children for tuberculous meningitis. Nevertheless, certain observations are of interest:

Survival

During the past five years 44 patients survived of 54 patients admitted to the hospital in all stages of illness, representing a survival rate of 81.5 per cent (table 4). It is interesting that the survival rate of the first year of therapy is essentially the same as this over-all rate, which compares very favorably with

TABLE 4

PERTINENT CLINICAL DATA OF 44 SURVIVING PATIENTS

Patient	Age	Sex	BCG	Days Ill	Extrameningeal Disease	Unconscious	Convulsions During Illness	Seizures After Illness	Present Electroencephalogram	Present Intellect
					First Year					
CW-1	6½ years	F	No	14	Left lung (miliary)	No	No	No	MDC (4 years)	High normal
FM-2	5½ years	F	No	22	Miliary	No	No	No	MDC (4 years)	Borderline
SL-3	6½ years	M	No	21	Left mediastinal nodes	7½ months	Severe	No	FC (5 years)	Normal
JL-4	7 years	M	No	8	Miliary	Yes	Mild	No (left hemiplegia)	FC (4 years)	Dull normal
RC-5	5 years	M	No	15	Right mediastinal nodes	Yes	Severe	No	FC (4 years)	High normal
CC-6	7 years	F	No	14	Right lung (upper lobe)	Semiconscious	Severe	No	FC (4 years)	Dull normal
BY-8	5 years	M	No	11	Left lung (lower lobe)	No	No	No	FC (4 years)	Normal
GL-9	7 years	M	?	17	Left lung (upper lobe)	3 months	Occasional focal	?	FC (3 months)	Deficient
RF-10	3½ years	M	No	18	Right mediastinal nodes	4 months	Moderately severe	No	Normal (3 years)	Normal
					Second Year					
DS-12	2½ years	F	No	14	None demonstrated	3 months	Severe	No	Normal (3 years)	Deficient
WK-13	6 years	F	No	15	Miliary	1 week	No	No	FC (2 years)	Normal
YR-14	8 years	F	No	14	Miliary	No	No	No	MDC (1 year)	Borderline
CM-16	20 months	M	No	10	Left lung (upper lobe)	1 week	Right spastic hemiplegia	No	FC (2 years)	Deficient
MT-18	3¾ years	M	No	23	Miliary	1 week	No	No	Normal (2 years)	Dull normal
MM-19	11 years	M	No	12	Left mediastinal node	1 week	No	No	FC (2 years)	Dull normal
					Third Year					
HJ-22	5 years	F	No	11	Right lung	2 weeks	One generalized seizure at onset	No	FC (2 years)	Borderline
PS-23	5 years	F	No	5	Miliary	No	No	No	MDC (2 years)	Dull normal
AS-24	4 years	M	Yes	14	None demonstrated	1 year	Severe	Yes	SDC (2 months)	Deficient
YP-26	10 years	M	No	9	Right mediastinal nodes	3 weeks	Moderately severe	No	FC (2 years)	Dull normal
JB-28	6 years	M	No	41	None demonstrated	1 year	Severe	No	SDC (2 years)	Deficient
ML-29	10 months	F	No	23	Right lung (lower lobe)	No	Moderately severe	No	SDC (2 years)	Deficient
RL-30	3 years	M	No	21	Left mediastinal nodes	1 month	Moderately severe	Yes	SDC (1 year)	Deficient
YD-31	12 years	M	No	12	Left mediastinal nodes	1 week	No	No	MDC (1 year)	High normal
LP-32	12 years	F	No	8	Miliary	No	No	No	FC (1 year)	Borderline
BR-34	5 years	F	No	14	Miliary	No	No	No	SD (1 year)	Deficient
DA-35	3 years	M	No	11	Right lung (lower lobe)	No	No	No	SD (1 year)	Dull normal

					Fourth Year					
RB-37	7 months	M	No	10	? Right mediastinal nodes	1 month	Moderately severe	No	SDC (1 year)	Deficient
LC-38	4 years	M	No	21	Right lung (lower lobe)	No	No	No	Normal (1 year)	High normal
JM-39	8 years	M	No	47	Miliary	Semiconscious, 1 week	No	No	FC (1 year)	Dull normal
GW-40	14 years	M	No	9	Miliary	No	No	No	SD (1 year)	Dull normal
ES-41	12 years	M	No	13	Miliary	No	No	No	MDC (1 year)	Borderline
AW-42	5 years	F	No	21	None demonstrated	semiconscious, 1 week	No	No	FC (1 year)	Borderline
RA-43	5 months	F	No	14	Miliary ?	1 month	Focal	?	SDC (1 year)	Deficient
WP-44	4½ years	M	No	10	Miliary	semiconscious, 1 week	No	No	MDC (1 year)	Normal
JU-45	3 years	F	No	27	None demonstrated	No	No	No	MDC (1 year)	Borderline
JE-46	7 years	M	No	14	Miliary	1 month	No	No	SD (1 year)	Normal
FS-47	3 years	F	Yes	7	Miliary	No	No	No	SD (1 year)	Deficient
GL-48	2 years	F	No	21	Miliary	No	No	No	FC (1 year)	Normal
LF-49	4½ years	F	No	14	Miliary	No	No	No	Normal (1 year)	Normal
					Fifth Year					
FP-50	16 months	M	No	16	Right lung (middle lobe)	No	Focal	Yes	MDC (6 months)	Deficient
PR-51	8 years	F	No	7	None demonstrated	No	No	No	Normal (6 months)	Dull normal
JT-52	13 years	F	No	21	Miliary	No	No	No	MDC (6 months)	Normal
LB-53	2 years	F	No	19	Miliary	No	No	No	Normal (1 year)	Dull normal ?
ML-54	19 months	M	Yes	8	None demonstrated	No	No	No	MDC (6 months)	Deficient

Electroencephalographic changes: Normal; FC—focal changes; MDC—mild diffuse changes; SDC—severe diffuse changes; SD—subcortical dysfunction.

Time after therapy is indicated in parentheses. Plan of therapy used for any patient may be seen by referring to table 2.

the survival rates presented by other investigators. It is pointed out and emphasized that no claim is made to show that the method of treatment used by the present writers is in any way superior to the methods used elsewhere. They are convinced that adequate therapy in tuberculous meningitis is not the result of antimicrobial therapy alone, important as it is; but adequate therapy is effected by a combination of procedures, dependent on the severity of the illness, not the least being adequate control of intracranial pressure. An unconscious and severely ill patient with tuberculous meningitis presents an entirely different problem from a patient who is conscious and mildly ill. In the former case it is believed that removal of blocking exudate at the base of the brain is vital to survival, whereas in the latter instance antimicrobial therapy alone may suffice.

Sequelae

Reference to table 2 reveals the incidence of sequelae that were encountered during treatment of this disease, viz., gross deafness, orthopedic deformities, optic atrophy, vestibular damage, and intellectual disturbances of varying severity. Reference has been made to their elimination. Those sequelae revealed by electroencephalography and psychometric examination have been of particular interest.

Electroencephalography: Electroencephalograms have been made early in each patient's illness, at the termination of therapy and, if possible, annually thereafter. It was observed that more frequent electroencephalograms revealed little useful information and that significant changes were more apparent if these were evaluated in a long-term study. The following general observations are made:

Four initially treated patients all had minor electroencephalographic changes at the end of therapy, and the electroencephalograms of 2 children subsequently became normal. Nevertheless, one of these normal electroencephalograms became abnormal at a later date.

Five children had normal electroencephalograms during the initial stages of their illnesses and all 5 were normal at the end of therapy. Nevertheless, 4 of them subsequently exhibited some degree of abnormality.

Ten children exhibited "mild diffuse changes" only during the initial stages of their illnesses; 9 of these 10 children exhibited further improvement at the end of therapy; and 4 of them subsequently exhibited normal electroencephalograms. Nevertheless, 3 of the 4 children with subsequent normal electroencephalograms had a reversion to some degree of abnormality at a later period.

Twenty children exhibited severe diffuse changes during the initial stages of illness and 18 of these children exhibited improvement in their electroencephalograms at the termination of therapy. Two of the children failed to exhibit any improvement whatsoever. Two patients who had improved electroencephalograms at the end of therapy eventually were observed to have normal electroencephalograms, but one of these 2 children later exhibited some regressive changes. One other child who had improved initially later proceeded to exhibit extensive abnormalities.

Three patients exhibited severe focal changes and all had improved at the end of therapy; the electroencephalogram of one of them subsequently became normal,

whereas the electroencephalogram of one other who had improved initially later showed regressive changes.

One patient initially exhibited evidence of subcortical damage, whereas another patient exhibited evidence of brain-stem damage and follow-up electroencephalograms revealed no significant changes.

The following table summarizes the present status of the electroencephalograms of the 44 patients who have been followed for periods varying from one to five years in relation to their state of consciousness at the time of admission.

Present Electroencephalogram	Conscious	Unconscious
Normal	4	3
Focal changes	4	10
Mild diffuse changes	9	3
Severe diffuse changes	—	6
Subcortical dysfunction	4	1

It is evident from the above observations that the majority of children (88.6 per cent) have abnormal electroencephalograms some time during the active phase of tuberculous meningitis. Furthermore, the majority of these electroencephalograms remain abnormal, with few exceptions. There appears to be, for the most part, a direct correlation between the severity of the disease and the electroencephalographic disturbances observed, the state of consciousness on admission being used as an indicator of severity. Nevertheless, 2 of the children who were unconscious now have normal electroencephalograms although, surprisingly, one is mentally deficient. These electroencephalographic findings will be presented in greater detail in a subsequent communication.

Psychometric evaluation: Psychometric evaluation was attempted under circumstances as carefully controlled as possible; the same two psychologists examined all children who were either English-speaking, French-speaking, or Eskimo. The children of similar age groups "lived-in" in a specially prepared ward where interviews were held. A preliminary interview established contact with the child through playing and talking, and at a second interview the psychometric tests were given, the chief ones being the Wechsler Intelligence Scale for Children, the Merrill-Palmer Test, the Bender-Gestalt Test, and the Draw-a-Person Test. In addition, the parents were interviewed in an attempt to add further information on the child's abilities prior to his illness. Furthermore, during the interview with the parents an attempt was made to assess the general intellectual capacities of the parents themselves. The various ages of the relatively small number of children tested, their different socio-economic backgrounds, the variation in degree of severity of their illnesses, the various periods of hospitalization, and the corresponding lack of and variation in school attendance, all made it impossible to give exact statistical and numerical evaluation of the intellect of these children. Furthermore, and of particular importance, it was found that the abilities of these children were scattered and that there often appeared to be selective intellectual damage.

The following table shows the approximate intelligence quotients of these children and the relative states of consciousness at the time of admission:

I.Q.	Conscious (45.4%)	Unconscious (54.6%)
High normal	2	2
Normal	5	4
Dull normal	5	6
Borderline normal	6	1
Retarded	3	10

These results indicate that the majority apparently had intellectual capacities that fell within "normal limits." It is interesting that the four "high average" children came from better social environments than the majority of the other children and that 6 of the 7 "borderline" cases had cultural factors involved, 2 being Eskimos. A third Eskimo child in the retarded group would not impress one as being retarded. In the deficient groups, all of the children, except 3, are able to play and enjoy their homes, the 3 exceptions being severely retarded.

The results of the tests need further clarification, but it is obvious from clinical, electroencephalographic, and psychometric examinations that all of the children have suffered some degree of cerebral injury. Contributing factors to this most interesting aspect of the disease which are not considered here, but which must be given greater consideration, are: the age of onset of the disease; the severity of clinical symptoms; the day the disease was diagnosed and adequate therapy begun; social and cultural factors; and variations in treatment.

Mortality: A mortality rate of 18.5 per cent occurred among these patients. Nevertheless, it is misleading to conclude that therapy failed in all of the 10 patients and that they died of tuberculous meningitis; such was not the case.

TABLE 5.4

SIGNIFICANT FACTORS TO BE CONSIDERED IN DEATHS OF TEN PATIENTS

Patient	Age	Sex	BCG	Days Ill	Extrameningeal Disease	Unconscious	Convulsions	Day of Death After Onset of Therapy
AG-7	1 year	F	No	30	Pulmonary	No	No	48
JF-11	14 years	M	No	60+	Miliary	2 months	No	13
SV-15	4 years	F	No	37	Left lung (minimal)	Yes	Yes	53
MB-17	2½ years	M	No	16	Left lung (minimal)	Yes	Yes	9
MM-20	19 months	M	No	21	Miliary	Yes	Yes	40
CT-21	2½ years	F	No	15	Miliary	Yes	Yes	2
AN-25	4 years	F	No	4	Miliary	No	No	6
Ro.L-27	5 years	M	Yes	150	Miliary	Yes	Yes	78
DB-33	9 months	F	Yes	49	Miliary	No	Yes	251
BC-36	11 years	M	?	3	Far advanced pulmonary and renal; epilepsy	Yes (diabetic coma)	No	27

TABLE 5B

PATHOLOGY OF BRAIN

Patient	
AG-7	Extensive venous sinus thrombosis, secondary to acute dehydration; ventricular dilatation; microscopic findings characteristic of tuberculous meningitis.
JF-11	Extensive subarachnoid hemorrhage secondary to "burr holes" in skull; cystic degeneration of right temporal, parietal and occipital lobes; extreme ventricular dilatation; microscopic lesions typical of tuberculosis.
SV-15	Herniation of brain through "burr holes" in skull and other signs of acute increase in intracranial pressure.
MB-17	Flattening of gyri; no evidence of damage in region of brain stem. (Death coincidental with cisternal puncture.)
MM-20	Flattening of gyri and obliteration of sulci; abundant basal exudate extends into and fills fourth ventricle; thrombosis of vertebral artery.
CT-21	Advanced state of deterioration on admission; died on second hospital day with signs of increased intracranial pressure. No autopsy granted.
AN-25	Generalized extensive miliary disease with multiple miliary tubercles over brain and spinal cord.
Ro.L-27	Rupture of large tuberculoma of the cerebellum and hemorrhage into the floor of the fourth ventricle; extreme dilatation of ventricular system.
BC-36	Extensive subarachnoid exudate; sectioning revealed foci of necrosis throughout brain substance, apparently on a vascular basis (massive fibrocaseous lesions of lungs), and diabetic coma.
DB-33	Filling and obstruction of fourth ventrical with exudate associated with extreme dilatation of ventricular system.

Furthermore, it is emphasized that *all* patients were treated regardless of their state of illness on admission, and all deaths are included, irrespective of the time when they occurred following admission. In table 5A are illustrated significant factors that should be considered in the 10 patients who died, in 6 of whom death had little direct relationship to tuberculous meningitis. The following are examples:

AG-7, an infant, died of an extensive cerebral venous thrombosis associated with severe dehydration.

JF-11 was hopelessly ill on admission, and his death, following shortly after burr holes were made in his skull, was obviously inevitable as revealed by the far advanced disease in his brain. No treatment in this case could have succeeded.

AN-25 had such extensive and severe cerebrospinal tuberculous meningitis associated with miliary disease of great toxicity that it would be unreasonable to expect recovery in such advanced disease.

RoL-27 had been treated elsewhere for a period of six months and was admitted to the hospital in an advanced state of deterioration. He did not respond to therapy and at autopsy advanced disease of the brain was present to such a degree that recovery could not conceivably have been expected.

BC-36 was a hopelessly ill patient, with uncontrolled diabetes (coma), epilepsy, renal tuberculosis, and extensive fibrocaseous disease of the lungs.

CT-21 was admitted to the hospital in a hopelessly deteriorated state and died two days later; any form of therapy would have been most unlikely to effect recovery.

Therapy could have been expected to succeed in 4 other patients who died:

MM-20 was seriously ill with both miliary and meningeal disease, and it is recognized that he died subsequent to uncontrolled intracranial pressure. Thrombosis of a vertebral artery probably contributed to this infant's demise.

SV-15. This child, although seriously ill, died, it is believed, from uncontrolled intracranial pressure.

MB-17. This boy died unexpectedly, coincidentally with a cisternal puncture, and it is likely that this procedure played some part in his death.

DB-33 represents outright failure of therapy, for meningitis was diagnosed by bacteriologic culture before clinical signs were evident. Several factors undoubtedly contributed to her death, including her young age, too small doses of streptomycin, and uncontrolled intracranial pressure, the results of which were evident at autopsy.

It is evident that the majority of deaths occurred in patients ill with such severe brain damage or disease elsewhere that no treatment could reasonably have been expected to succeed. Therefore, in evaluating mortality due to tuberculous meningitis and its relationship to therapy, it would be incorrect to attribute these deaths to tuberculous meningitis. Allowance for this should be made in evaluating the efficacy of therapy or its failure.

It would be remiss not to emphasize the great importance of persisting with therapy when the outlook for survival appears to be hopeless. In at least 3 patients, unconscious from six to fourteen months and for whom there appeared to be absolutely no hope, therapy was continued, and these children were observed to progress over the past few years from a state of prolonged coma and vegetation to one of unexpected recovery with remarkably little intellectual impairment.

It is believed that death in 4 patients was a direct result of unrelieved intracranial pressure. On many occasions prompt improvement in patients when intracranial pressure has been reduced has been impressive. This has usually been effected by lumbar or cisternal puncture, and occasionally by an in-dwelling polyethylene ventricular drain.

Pathologic Findings

Permission for autopsy was granted in 9 of the 10 patients who died. The most striking gross lesions are listed on table 5*B*. It is evident that in nearly all instances the pathology consisted of damage caused by excessive intracranial pressure as evidenced by flattened gyri, narrowed sulci, and varying degrees of ventricular dilatation, with loss of brain substance. Another prominent feature was vascular occlusion noted both grossly and microscopically, with associated destruction of brain substance varying in size from multiple microscopic foci to gross infarction of the entire lobes. Microscopically, the findings in each case were characteristic of tuberculosis of the central nervous system.

These pathologic findings obviously are not presented as evidence for the efficacy of PPD in the removal of blocking exudate at the base of the brain, for it was found to be exceedingly difficult to demonstrate any such evidence. They are presented rather to indicate the degree of disease that must be accepted as having been present and to permit one to judge fairly of the possibilities of successful therapy at the time that it was initiated.

Reasonable clinical, bacteriologic, and pathologic evidence for the efficacy of PPD in the treatment of tuberculous meningitis has been presented elsewhere (2, 8–12). Further evidence is presented later on in this report.

Bacteriologic Findings

The bacteriologic results have been published in detail elsewhere (12) and only a brief summary will be given.

Specimens of cerebrospinal fluid were sent to the laboratory before treatment and usually every day for the first forty days of therapy; thereafter they were sent every second day for the next one hundred and sixty days. A specimen was received from the patients who were discharged from the hospital every two weeks for the first two months, and thereafter once every four months.

At the beginning of this work, specimens of cerebrospinal fluids were received from 16 patients and were cultured directly on Löwenstein medium slopes (table 6). The diagnosis was confirmed bacteriologically in only 13 patients by this method of culture. (In the remaining 3 patients the diagnosis was confirmed by direct smear at the time of admission to the hospital and at autopsy.) Tubercle bacilli were recovered before or during the earliest stages of intrathecal

TABLE 6

BACTERIOLOGIC RESULTS IN PATIENTS WHOSE CEREBROSPINAL FLUIDS WERE CULTURED DIRECTLY ON LÖWENSTEIN MEDIUM SLOPES

Patient	Total Number of Specimens of Cerebrospinal Fluid Tested	Total Number of Cultures Positive for *Mycobacterium Tuberculosis*
CW-1	66	2
FM-2	71	1
SL-3	57	1
JML-4	53	1
RC-5	52	1
CC-6	49	1
AG-7	7	1 (died)
BY-8	46	1
GL-9	66	None*
RF-10	57	None*
JF-11	4	None (died)
DS-12	77	1
WK-13	77	1
YR-14	64	1
SV-15	39	1 (died)
CM-16	73	1

* One specimen of cerebrospinal fluid showed acid-alcohol fast rods, on direct smear, before patient's admission to the hospital.

TABLE 7

BACTERIOLOGIC RESULTS IN PATIENTS WHOSE CEREBROSPINAL FLUIDS WERE CULTURED ON MEMBRANE FILTER

Patient	Total Number of Cultures Positive for *Mycobacterium Tuberculosis*	Period of Treatment in Which the Last Culture Positive for *Mycobacterium Tuberculosis* Was Obtained
MB-17	4	First week of the first course (died)
MT-18	10	Fourth week of the second course
MM-19	3	First week of the first course
MM-20	4	Second week of the first course (died)
CT-21	2	First week of the first course (died)
HJ-22	1	Positive only before treatment
PS-23	3	Second week of the first course
AS-24	None	
AN-25	5	First week of the first course (died)
YP-26	None*	
Ro.L-27	4	Sixth week of the first course (died)
JB-28	15	Second week of the second course
ML-29	11	Sixth week of the first course
Ri.L-30	8	Second week of the first course
YD-31	9	Third week of the first course
LP-32	2	First week of the first course
DB-33	35	Seventh month of treatment (died)
BR-34	21	Sixth week of the first course
DA-35	21	Second week of the second course
BC-36	17	Third week of the first course (died)
RB-37	18	Sixth week of the first course
LC-38	2	Positive only before treatment
JM-39	23	Sixth week of the first course
GW-40	1	First week of the first course
ES-41	9	Third week of the first course
AW-42	3	Second week of the first course
RA-43	27	Fifth month of treatment
WP-44	4	Second week of the first course
JV-45	9	Fifth week of the first course
JE-46	7	First week of the first course
FS-47	1	Positive only before treatment
GG-48	3	First week of the first course
LF-49	5	Second week of the first course
FP-50	2	First week of the first course
PR-51	12	Second week of the first course
JT-52	2	Positive only before treatment
LB-53	18	Second week of the second course
ML-54	4	Fifth week of the first course

* An unidentifiable acid-fast organism was isolated from one specimen.

treatment only, and the average number of cultures positive for *M. tuberculosis* was less than two per patient.

Failure to culture *Mycobacterium tuberculosis* by this method led to an attempt to recover tubercle bacilli from cerebrospinal fluids by filtration technique. By this method of filtration technique, bacteriologic diagnosis was

established in 36 of 38 patients (table 7). From many patients *Mycobacterium tuberculosis* was repeatedly isolated and for long periods of time during treatment. *Mycobacterium tuberculosis* became resistant to streptomycin in only one patient (*RoL-27*).

Correlation between the incidence of cultures positive for *M. tuberculosis* and the reaction following PPD is difficult although tubercle bacilli were isolated before intrathecal PPD treatment from all but 4 of the patients. Nevertheless, specimens of cerebrospinal fluids from these 4 patients showed growth of *Mycobacterium tuberculosis only* after administration of PPD. Further reference will be made to the liberation of *Mycobacterium tuberculosis* into the cerebrospinal fluid after PPD reaction.

Discussion

A unique and interesting feature of this report has been the use of PPD in a series of consecutive cases and for this reason this comment will deal primarily with the use of PPD. It has been pointed out that PPD has received a limited clinical trial, for it appears that its use has been feared without an adequate clinical trial. The experience of the present writers with the use of this substance has been highly favorable, and it is believed that all opinions based on adequate clinical trial should be heard. In respect to safety, it has been found that if this substance is employed with caution and good clinical judgment, it is unwarranted to consider PPD highly dangerous. The rationale for the use of PPD, as originally presented by Smith and Vollum is logical, for it is generally agreed that alteration or removal of the blocking tuberculous exudate in the basal cistern of the brain, permitting free flow of cerebrospinal fluid, is fundamental to recovery; to this end PPD was employed. Better still would be the prevention of an accumulation of exudate at the base of the brain, and the rationale for the use of PPD would still hold in this respect as well.

The efficacy of PPD to produce alteration and eventual removal of the exudate in tuberculous meningitis is almost impossible to prove in living human patients. Nevertheless, the following observations of the present writers are noteworthy:

First, there was the revolutionary experience of an unusually high survival and recovery rate during the first year of PPD therapy at the Alexandra Hospital when 9 of 11 patients survived; the two deaths that occurred were not due directly to tuberculous meningitis. This recovery rate, as noted in table 3, was considerably higher than the recovery rate when similar therapy was employed without PPD.

Second, there were the "showers" of colonies of *M. tuberculosis* that were cultured from the cerebrospinal fluid following a clinical reaction to PPD. This was not a common finding, yet it was striking when it did occur, and there is no other reasonable explanation for the observation than that a hypersensitivity inflammatory reaction to PPD caused an alteration of the exudate at the base of the brain with liberation of these organisms. At no time other than after a clinical response to PPD were these "showers" of organisms observed.

Third, there was the actual disappearance of a block to the free flow of cerebrospinal fluid observed in several instances but best illustrated in the case of *JB-28*, a six-year-old boy, hopelessly ill for two months and apparently about to die. Although cerebrospinal fluid could always be removed, up to 75 ml. a day by three to four lumbar punctures, it became impossible to control the intracranial pressure by this means. An in-dwelling polyethylene ventricular drain was employed, from which 150 to 230 ml. of cerebrospinal fluid drained each day under normal cerebrospinal fluid pressure, and in this manner the intracranial pressure was effectively controlled. During the first few weeks after the introduction of this ventricular drain, obstruction of the tube was associated with prompt increase in intracranial pressure. Intensive PPD therapy was instituted and maintained in this patient, and eventually it was observed that the quantity of cerebrospinal fluid draining from the ventricles gradually lessened. At the end of four months the tube was removed, whereupon it was observed that the intracranial pressure remained at a normal level, the return to normal flow of cerebrospinal fluids being attributed to the removal of the blocking exudate at the base of the brain associated with the administration of PPD. From these observations, therefore, it would appear reasonable to suggest that PPD had played an important and beneficial role in the therapy of these patients.

In table 8 the results of therapy are illustrated in relatively recent groups of patients when isoniazid was used in combination with streptomycin and other antituberculous drugs. At the present time this combination of antimicrobial therapy appears to be the one of choice in tuberculous meningitis, and again the results of therapy observed in this Unit over the past five years compare favorably with them.

The results of Torres-Gost (16) are not included, as was done by Weinstein and Meade, for it is believed that they are open to some criticism. No bacteriologic studies were made and cerebrospinal fluid studies were on the basis of cell counts only. Duration of therapy was remarkably brief, three months. Although 75 per cent of these patients were stuporous or comatose at the time of the institution of therapy, it was most unusual that there were no intellectual sequelae, the chief complications being paresis of the anterior tibial muscles.

TABLE 8*

RESULTS OF THERAPY WITH ISONIAZID COMBINED WITH OTHER ANTIMICROBIALS

Investigator	Number of Patients	Number Living	Per Cent Survival
Des Autels (3)	16	14	87.5
Anderson (13)	7	7	100.0
Waddell (14)	6	4	66.7
Lorber (15)†	38	28	73.7
Totals	67	47	70.1

* Modified after Weinstein and Meade (1).

† Lorber observed that isoniazid added to streptomycin and PAS therapy was no more efficacious than when streptomycin and PAS were used together.

A recent report by Shane and Riley (17) of the employment of cortisone in conjunction with tuberculostatic drugs has met with unexpectedly good results in spite of apparent contraindications in the therapy of tuberculosis. The rationale for the use of cortisone in tuberculous meningitis is that on theoretic grounds it might be thought to lessen the inflammatory reaction, preventing the formation of blocking exudate at the base of the brain.

At the present, it would seem that there is no unique method of therapy for tuberculous meningitis, from which all patients may be expected to recover. A one hundred per cent survival and recovery rate is a highly elusive figure and it is made almost impossible over a period of time by the patients who are admitted in a hopeless state of deterioration after a late diagnosis. Therapy would seem to be a selective affair. The majority of patients who are conscious and not severely ill may be expected to recover with isoniazid-streptomycin therapy, the use of intrathecal streptomycin apparently being unnecessary in most cases. Nevertheless, it is obvious that antimicrobial drugs alone, essential as they are, cannot be expected to effect recovery in all cases. There are other factors to be considered, particularly in the severely ill patient who is unconscious and has increased intracranial pressure. It is emphasized that adequate control of intracranial pressure, so easily overlooked at times, is essential to survival of these patients and it is the opinion of the present writers that PPD should be employed in this type of patient.

Summary

Fifty-four patients with tuberculous meningitis have been treated with varying combinations of antituberculous drugs and PPD over a five-year period, with a survival rate of 81.5 per cent. Sequelae encountered have been deafness and loss of vestibular function, orthopedic deformities, atrophy of the optic nerve and intellectual impairment, all of which may be minimized and perhaps eliminated by proper therapeutic measures.

Psychometric evaluation of the 44 surviving children indicates that approximately two-thirds have intelligence that may be considered within normal limits, the remaining children being considered defective in varying degrees. Electroencephalographic disturbances were noted in all but one child at some stage during the active illness or in subsequent follow-up examinations. In only 7 children did the electroencephalogram return to normal during one to four years of follow-up observations.

Particular emphasis is placed on the importance of controlling intracranial pressure during the early months of therapy, not only to lessen cerebral damage but to reduce mortality during this period. Furthermore, the importance of perseverance in therapy is emphasized when prognosis would appear to be hopeless, for unexpected and dramatic recovery has been observed to occur.

Pertinent bacteriologic and pathologic findings are presented. The role of PPD in therapy of these patients with tuberculous meningitis has been discussed, and the opinion is held, after a fair and careful clinical trial, that this substance has been found to be effective in the therapy of tuberculous meningitis; the results compare favorably with those of other investigators.

Sumario

Meningitis Tuberculosa

Cincuenta y cuatro enfermos con meningitis tuberculosa fueron tratados con varias combinaciones de agentes tuberculostáticos y PPD durante un período de cinco años con una tasa de 81.5 por ciento de sobrevivencia. Las secuelas observadas fueron sordera y pérdida de la función vestibular, deformidades ortopédicas, atrofia del nervio óptico y deterioro intelectual, todo lo cual puede eliminarse o reducirse al mínimo con un cálculo terapéutico acertado.

La justipreciación psicométrica de los 44 niños sobrevivientes indica que aproximadamente dos terceras partes tienen inteligencia que puede clasificarse dentro de límites normales, considerándose al resto de los niños como más o menos defectuosos. En todos los niños, menos uno, notáronse pertrurbaciones electroencefalográficas en algún período de la enfermedad activa o en los exámenes subsiguientes de observación, y al cabo de uno a cuatro años de observación subsiguiente sólo en 7 niños ha vuelto el encefalograma a lo normal.

Hácese hincapié en particular en la gran importancia que reviste la regulación de la presión intracraneal durante los primeros meses de la terapéutica, no tan sólo para aminorar la lesión cerebral sino para reducir la mortalidad durante este período. Además, recálcase la mucha importancia de la perseverancia en la terapéutica cuando el pronóstico parece desesperado, pues se han observado curaciones inesperadas y dramáticas.

Preséntanse pertinentes hallazgos bacteriológicos e histopatológicos. Discutiendo el papel del PPD en el tratamiento de estos enfermos, se sostiene que, después de una prueba imparcial y cuidadosa, esta substancia se ha mostrado eficaz en el tratamiento de dicha enfermedad, comparándose los resultados favorablemente con los obtenidos por otros investigadores.

Resume

Méningite tuberculeuse

Le traitement de 54 patients atteints de méningite tuberculeuse a été effectué au moyen de différentes combinaisons d'agents tuberculostatiques et de PPD pendant une période de cinq ans en donnant un taux de survie de 81,5%. Les séquelles rencontrées comprenaient la surdité et la perte de la fonction vestibulaire, les déformations d'ordre orthopédique, l'atrophie du nerf optique et une déficience intellectuelle due à des lésions cérébrales. Toutes ces séquelles peuvent être éliminées ou minimisées par des mesures thérapeutiques appropriées.

L'évaluation psychométrique des 44 enfants survivants indique qu'approximativement deux tiers d'entre eux ont une intelligenc pouvant être considérée dans les limites normales, les autres enfants étant considérés comme déficients à des degrés divers. Les altérations du tracé électroencéphalographique ont été notées chez tous les enfants, sauf un, à un certain moment de la phase active de la maladie ou lors des examens qui ont suivi. L'électroencéphalogramme n'est redevenu normal que chez 7 enfants au bout de une à quatre années, au cours desquelles ces malades ont été suivis.

Les auteurs insistent particulièrement sur l'importance considérable du contrôle de la pression intracranienne durant les premiers mois du traitement, non seulement pour minimiser l'altération cérébrale, mais aussi pour réduire la mortalité au cours de cette période. De plus l'extrême importance de la persévérance dans le traitement, même lorsque le pronostic pourrait apparaître sans espoir, est mise en évidence, grâce aux guérisons spectaculaires et inattendues que l'on a observées.

Des résultats pertinents bactériologiques et anatomo-pathologiques sont présentés. Les auteurs commentent le rôle du PPD (tuberculine purifiée) dans le traitement de ces cas de méningite tuberculeuse et ils estiment, à la suite d'une expérimentation clinique objective et scrupuleuse, que cette substance s'est montrée efficace dans le traitement de cette

affection; les résultats soutiennent favorablement la comparaison avec ceux obtenus par d'autres investigateurs.

Acknowledgment

The writers wish to acknowledge the devoted nursing care given by Mrs. Mary Thornton and her staff, and the work of Dr. N. Freimanis and a large group of enthusiastic house physicians over the past five years, which has been an inestimably favorable factor in the treatment of the children described in this report.

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THE QUANTITATIVE DETERMINATION OF ISONIAZID AND PARA-AMINOSALICYLIC ACID IN BODY FLUIDS[1,2]

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INTRODUCTION

Shortly after the introduction of isoniazid as a chemotherapeutic agent in tuberculosis, Kelly and Poet (1) described a method for its estimation in biologic fluids. This method, with minor modifications introduced by Helmer (2), has been in routine use at this laboratory in clinical research for a number of years. In conjunction with this chemical method, a biologic method described by Morse and associates (3) has also been employed. The chemical method produced consistently higher results because it measures the total hydrazine-yielding metabolites of isoniazid plus the free hydrazide while the biologic method measures growth inhibition of *M. tuberculosis* in terms of an isoniazid standard. Hughes and her associates (4–6) studied the metabolism of isoniazid and observed gross variations in the mode of metabolism from one individual to another. The percentage of an administered dose of isoniazid recovered in the urine as free isoniazid ranged from 2.9 to 36.8, while the percentage recovered as the acetyl metabolite ranged from 14.1 to 73.9. The acetyl metabolite of isoniazid demonstrates no growth inhibition in the biologic assay. The hydrazone metabolites, alpha-ketoglutaric acid isonicotinoyl hydrazone and pyruvic acid isonicotinoyl hydrazone, do inhibit the growth of *M. tuberculosis*, as reported by Zamboni and Fachinelli (7) and confirmed by this laboratory. Whether this growth inhibition *in vitro* is produced by the hydrazone itself or by its breakdown in the test tube to release free isoniazid has not yet been established.

The need for a chemical method which can distinguish free isoniazid from therapeutically inactive metabolites in serum is clear. A number of methods reportedly specific for free isoniazid have appeared in the literature. A colorimetric method employing picryl chloride was described by Hunter (8). Deeb and Vitagliano (9) described a method employing a vanillin reagent. Investigation of this reagent by the present writers confirmed its specificity for isoniazid in the presence of its acetyl metabolite. Rubin and co-workers (10) were the first to attempt ultraviolet absorption as a means for estimating isoniazid in blood. They recognized its limitations, both with respect to sensitivity and specificity. The present writers have investigated the possibility of utilizing ultraviolet absorption as a means of estimating "total isoniazid" but observed

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that the background absorption in drug-free fluids from the same individual vary too widely for analytic purposes. Goldman (11) described a pH-engendered shift in the ultraviolet absorption maximum of isoniazid which required an unsubstituted hydrazide group and a free ring nitrogen. It has been observed by the present writers that both free isoniazid and the acetyl derivative shifted their absorption maxima to 300 millimicrons at pH 13, excluding the possibility of utilizing this method clinically.

Investigation of the solubility characteristics of isoniazid and *p*-aminosalicylic acid (PAS) revealed the possibility of separating these compounds by means of a simple extraction procedure. A vanillin reagent was then employed to measure the isoniazid and its two, bacteriologically active metabolites, pyruvic and alpha-ketoglutaric acid isonicotinoyl hydrazones. "Total isoniazid," viz., free plus conjugated metabolites including the acetyl derivative, was then measured by reaction between *p*-dimethylaminobenzaldehyde and the hydrazine obtained by acid hydrolysis. Free PAS was determined by direct reaction with *p*-dimethylaminobenzaldehyde before hydrolysis. Finally, total PAS, i.e., free plus conjugated metabolites, was determined in the same tube after mild hydrolysis. The intent of this paper is to describe the method developed from these studies and discuss its evaluation.

Method

The reagents employed were:

9N and 0.1N hydrochloric acid

0.1N sodium hydroxide

Anhydrous ammonium sulfate

Solvent solution: Three volumes of iso-amyl alcohol mixed with 7 volumes of ethylene dichloride

Purification of iso-amyl alcohol and ethylene dichloride: The iso-amyl alcohol was purified by shaking twice with an equal volume of 1N hydrochloric acid, once with 1N sodium hydroxide, and twice with distilled water. It was dried by shaking with anhydrous sodium sulfate and then filtered. Activated charcoal and sodium sulfate were added and filtered twice. The ethylene dichloride required only the drying and charcoal treatment.

Vanillin: The vanillin color reagent (4 per cent) was prepared by dissolving 1 gm. of vanillin in 25.0 ml. of absolute ethanol. The *p*-dimethylaminobenzaldehyde reagent was prepared by dissolving 0.3 gm. of *p*-dimethylaminobenzaldehyde in 5.0 ml. of absolute ethanol and 5.0 ml. of 9N hydrochloric acid.

Standardization: Concentrated aqueous solutions were prepared of isoniazid (1,000 γ per ml.), acetyl isoniazid (1,000 γ per ml.) and PAS (2,000 γ per ml.). These solutions were diluted with drug-free serum or urine to obtain the following concentrations: isoniazid, 0.5 to 15γ per ml. of serum and 1 to 30γ per ml. of urine; acetyl isoniazid, 0.5 to 10 γ per ml., PAS, 5 to 100 γ per ml. of serum and 100 to 300 γ per ml. of urine. These standard solutions were carried through the method described below and a Bouger-Beer Law curve was constructed for each of the three compounds.

Extraction Procedure

Ammonium sulfate, 3.2. gm., was weighed out into 50.0 ml. round-bottom, glass-stoppered, shaking bottles. Three milliliters of serum or 3.0 ml. of a 1:20 urine dilution were added and mixed by swirling. For the reference blank 3.0 ml. of distilled water was used and was carried through the entire procedure. Forty milliliters of the solvent solution were added and the bottles were stoppered tightly. The mixture was shaken

for thirty minutes horizontally and centrifuged for fifteen minutes at 3,000 r.p.m. Thirty-seven milliliters of the solvent solution were drawn off and transferred to round-bottom, glass-stoppered, shaking bottles, containing 4.0 ml. of 0.1N hydrochloric acid. These were shaken for fifteen minutes horizontally and centrifuged for fifteen minutes at 2,000 r.p.m. Three milliliters of the acid layer, were drawn off, placed in test tubes, and stoppered. Twenty-five milliliters of the organic solvent were drawn out and added to shaking bottles containing 4.0 ml. of 0.1N sodium hydroxide. These were shaken for fifteen minutes horizontally, and centrifuged for fifteen minutes at 2,000 r.p.m. Three milliliters of the aqueous base extract were transferred to 19 × 150 mm. cuvettes.

Determination of "free isoniazid": To 3.0 ml. of the acid extract, 0.2 ml. of vanillin color reagent was added, and the optical density of the acid vanillin solutions in the Beckman DU spectrophotometer was recorded at 365 millimicrons, the wave length of maximal absorption, using the blank as reference. The concentration of "free isoniazid" was read from the standard Bouger-Beer Law curve.

Determination of "total isoniazid": Three milliliters of the vanillin-containing solution were placed in 15.0 ml. graduated, centrifuge tubes and 1.5 ml. of the *p*-dimethylaminobenzaldehyde reagent were added. The tubes were placed in a boiling water bath for one hour, removed and cooled and then made up to 6.0 ml. with 0.1N hydrochloric acid. The solutions were transferred to 19 × 150 mm. cuvettes and the optical density was recorded at 450 millimicrons in the Coleman Jr. spectrophotometer using the blank as reference. On the standard Bouger-Beer Law curve for "total isoniazid," the optical density was found which corresponded to the concentration of "free isoniazid" determined above. This was subtracted from the optical density observed here for "total isoniazid" and the concentration of acetyl isoniazid was found which corresponded to this corrected optical density on the standard Bouger-Beer Law curve. This concentration of acetyl isoniazid was added to the concentration of free isoniazid determined above to obtain the concentration of "total isoniazid."

Determination of PAS: To 3.0 ml. of the aqueous basic extract in the 19 × 150 mm. cuvettes, 1.5 ml. of the *p*-dimethylaminobenzaldehyde reagent and 1.0 ml. of water were added. The optical density was recorded at 450 millimicrons using the blank as reference. The concentration corresponding to this optical density was found on the standard Bouger-Beer Law curve for PAS. These tubes were stoppered and transferred to a boiling water bath for thirty minutes. The optical density was recorded at 450 millimicrons again. The concentration corresponding to this optical density was found on the standard Bouger-Beer Law curve. This is the concentration of "total PAS," i.e., free plus conjugated PAS.

Results

The Bouger-Beer Law curve observed for the vanillin-isoniazid complex is presented in figure 1. The upper curve was obtained by carrying aqueous solutions of free isoniazid through the procedure described. The lower curve was obtained with solutions of free isoniazid in pooled human serum carried through the procedure. The difference in slope is referable to loss due to adsorption on the denatured serum proteins, since washing the precipitate results in better yields. No increase in reproducibility was obtained by washing the precipitate; hence it was decided to delete this step from the procedure. In the case of drug-free serum, the background optical density was very low.

In figure 2 may be seen the Bouger-Beer Law curve observed for the complex formed between *p*-dimethylaminobenzaldehyde and the hydrazine produced from the hydrolysis of acetyl isoniazid (upper curve) and the free hydrazide (lower curve) when pooled serum standard solutions are carried through the

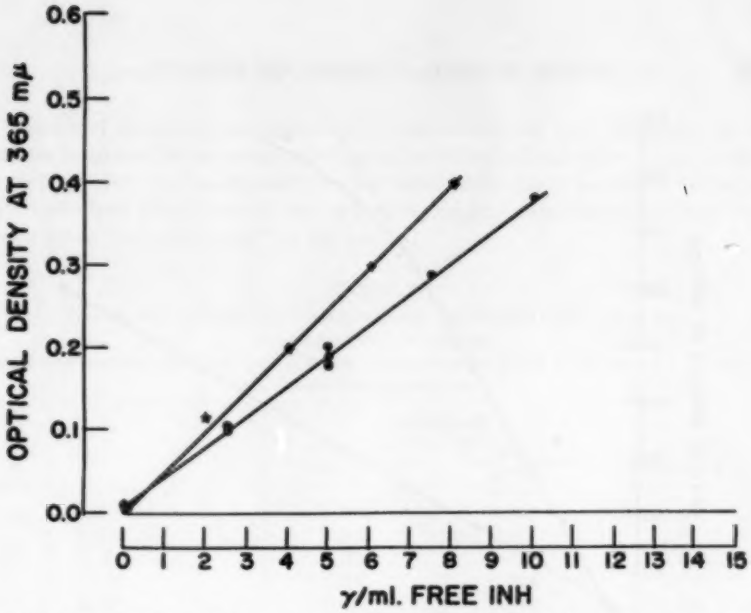

FIG. 1. The Bouger-Beer Law curve for the complex formed between vanillin and free isoniazid. The upper curve was obtained from aqueous solutions and the lower curve from serum solutions carried through the procedure described in the text.

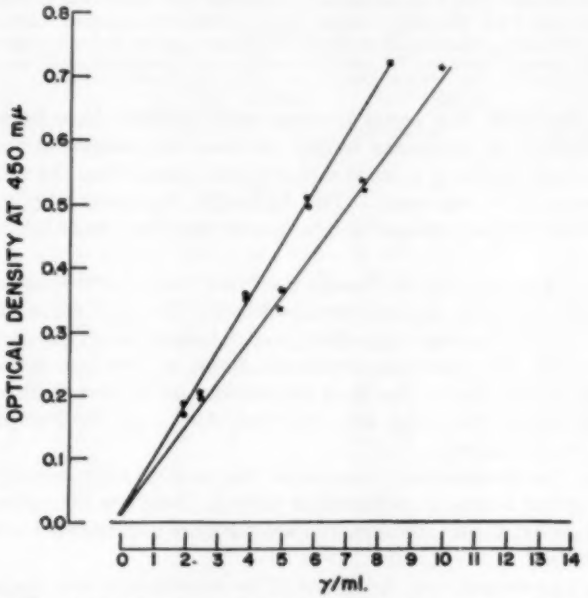

FIG. 2. The Bouger-Beer Law curve for the complex between *p*-dimethylaminobenzaldehyde and hydrazine from the hydrolysis of free and acetyl isoniazid. The upper curve is the acetyl isoniazid and the lower curve the free isoniazid in serum solutions carried through the procedure described in the text.

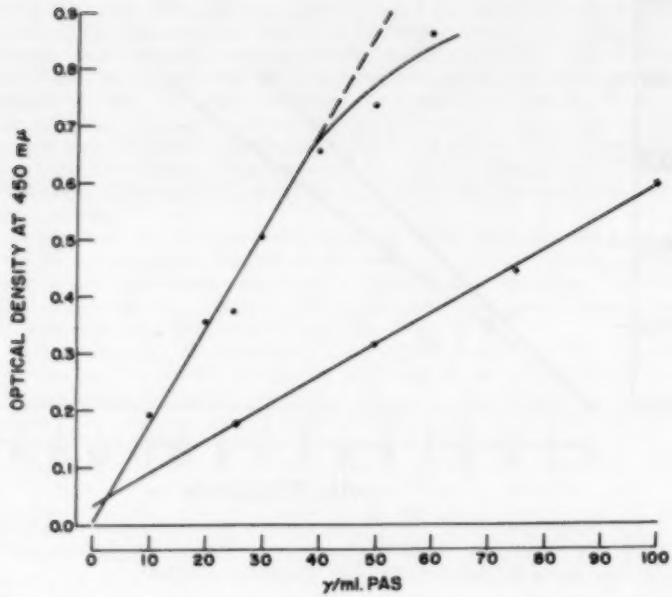

FIG. 3. The Bouger-Beer Law curve for the complex formed between *p*-dimethylaminobenzaldehyde and PAS. The upper curve was obtained with aqueous solutions and the lower curve with serum solutions of the drug carried through the procedure described in the text.

procedure described. The acetyl isoniazid concentrations have been corrected for the difference in molecular weight between this compound and the free hydrazide which results in a curve with a steeper slope. Thus the concentration of this compound is expressed as free hydrazide equivalent. Again the background optical density observed in the case of drug-free, pooled, human serum was low.

In figure 3 may be seen the Bouger-Beer Law curve for the complex formed between PAS and *p*-dimethylaminobenzaldehyde. The upper curve was obtained from aqueous and the lower curve from pooled, human serum standard solutions carried through the procedure described. Again, a very low optical density background in the case of the drug-free serum and a curve with a shallower slope in the case of the serum were observed, due to loss by adsorption on the denatured serum proteins.

The very low backgrounds observed in this method when applied to freshly drawn, drug-free serum are reflected in table 1. These are the optical densities actually observed in the procedure and are therefore uncorrected for the dilution factors involved.

In table 2 are summarized the results of the investigation into the fate of some of the metabolites of isoniazid and PAS by use of the method. The hydrazones of

isoniazid and the glycyl conjugate of PAS are not shown, since the purity of the samples employed in the study of these metabolites is in question. The complex formed between the vanillin and free isoniazid broke down to release hydrazine after hydrolysis which reacted with *p*-dimethylaminobenzaldehyde and was then measured as "total isoniazid" in the method.

TABLE 1

THE OPTICAL DENSITIES OBSERVED FOR DRUG-FREE SERA FROM SIX NONTUBERCULOUS DONORS

The optical density equivalent of a serum concentration of 0.5 γ of isoniazid per ml. is shown for comparison

	Optical Density		
	"Free" Isoniazid	"Total" Isoniazid as "Free"	*p*-Aminosalicylic Acid
J. C. (1)	0.002	0.009	0.007
J. C. (2)	0.009	0.007	—
E. J.	0.009	0.010	0.010
W. M.	0.007	0.014	0.002
B. M.	0.009	0.010	0.004
D. S.	0.008	0.012	0.001
J. M.	0.029	0.008	—
0.5γ/ml.	0.018	0.043	0.0029

TABLE 2

THE FATE OF SOME METABOLITES OF ISONIAZID AND PAS IN THE METHOD DESCRIBED

Drug	Solvent	"Free" Isoniazid			"Total" Isoniazid			PAS		
		Concentration in γ/ml		Per Cent Recovery	Concentration in γ/ml		Per Cent Recovery	Concentration in γ/ml		Per Cent Recovery
		Theoretical	Found		Theoretical	Found		Theoretical	Found	
Isoniazid	Water	5.0	4.64	92.8	5.0	4.76	95.2	0	0	0
	1–20 urine	5.0	4.74	94.8	5.0	4.88	97.6	0	0	0
	Serum	5.0	4.55	91.0	5.0	4.45	89.0	0	0	0
Acetyl isoniazid*	Water	3.5	0	0	3.5	3.16	90.3	0	0	0
	1–20 urine	3.5	0	0	3.5	3.31	94.4	0	0	0
	Serum	3.5	0	0	3.5	3.00	85.7	0	0	0
Isonicotinic acid	Water	10.0	0	0	10.0	0	0	0	0	0
	Serum	10.0	0	0	10.0	0	0	0	0	0
Sodium *p*-amino salicylate dihydrate†	Water	0	0	0	0	0	0	25	22.0	88.0
	1–20 urine	0	0	0	0	0	0	25	22.5	90.0
	Serum	0	0	0	0	0	0	25	21.5	86.0
Sodium *p*-acetamino salicylate monohydrate†	Water	0	0	0	0	0	0	10	0	0
	1–20 urine	0	0	0	0	0	0	10	0	0
	Serum	0	0	0	0	0	0	10	0	0

* As isoniazid.

† As PAS.

TABLE 3

SERUM CONCENTRATIONS OBSERVED AMONG A GROUP OF NONTUBERCULOUS VOLUNTEERS AT TWO AND SIX HOURS AFTER THE ADMINISTRATION OF 10 MG. PER KG. PER DAY OF ISONIAZID

	Isoniazid in γ per Ml.							
	"Free"				Total			
	August 2, 1956		August 7, 1956		August 2, 1956		August 7, 1956	
	2 Hours	6 Hours	2 Hours	6 Hours	2 Hours	6 Hours	2 Hours	6 Hours
J. C.	2.2	0.8	3.4	1.3	3.8	2.4	5.9	2.9
E. J.	0.7	0.1	1.3	0.03	3.4	1.4	3.9	2.1
F. K.	2.5	1.0	3.1	1.2	3.4	1.6	4.2	1.9
H. K.	3.0	1.3	4.1	0.8	4.0	2.2	8.9	2.5
J. M.	2.0	1.6	1.2	0.7	3.1	2.9	4.8	2.1
H. S.	0.6	0.0	0.7	0.3	2.9	1.2	3.3	1.8
R. T.	0.8	0.0	0.7	0.1	2.6	1.2	2.8	1.5
R. W.	1.7	0.7	2.3	1.0	2.5	1.8	4.0	1.5
O. W.	1.2	0.0	0.9	0.0	3.4	2.0	3.6	1.5
P. W.	2.4	0.9	2.2	1.8	3.4	1.7	6.2	4.4

TABLE 4

BLOOD CONCENTRATIONS OF ISONIAZID AND *p*-AMINOSALICYLIC ACID AT TWO-, THREE-, AND SIX-HOUR INTERVALS AFTER ADMINISTRATION TO TUBERCULOUS PATIENTS

Dose: Isoniazid 10 mg. per kg. per day; parasal, 12 gm. per day; PAS-C, 6 gm. per day.

Isoniazid and Parasal					Isoniazid and PAS-C				
Patient	Time	in γ per ml.			Patient	Time	in γ per ml		
		"Free" Isoniazid	"Total" Isoniazid	*p*-Amino-salicylic Acid			"Free" Isoniazid	"Total" Isoniazid	*p*-Amino-salicylic Acid
	hours					*hours*			
A	2	5.1	6.9	104	J	2	1.3	3.6	43
	3	2.8	4.2	83		3	1.0	3.0	20
	6	2.2	3.6	30		6	0	1.7	0
B	2	3.1	6.5	79	K	2	3.6	6.0	17
	3	3.1	5.4	52		3	5.1	7.1	9
	6	1.4	3.7	7		6	3.9	5.5	2
C	2	4.4	6.9	52	L	2	6.4	7.9	38
	3	3.9	5.1	32		3	5.4	7.6	27
	6	2.7	4.5	14		6	5.0	5.8	4
D	2	5.5	8.5	106	M	2	5.7	7.8	26
	3	5.5	7.6	90		3	4.5	7.1	8
	6	2.8	5.1	39		6	1.4	4.7	0
E	2	4.4	6.4	59	N	2	1.2	5.4	43
	3	3.5	5.7	56		3	0.5	3.1	27
	6	2.5	4.2	25		6	0	3.3	4
F	2	1.3	4.4	83	O	2	1.6	5.0	23
	3	0.8	3.2	90		3	0.9	4.6	17
	6	0.8	2.6	17		6	0.4	3.3	5
G	2	5.0	8.2	111	P	2	6.2	7.5	33
	3	3.0	5.7	101		3	5.5	6.8	34
	6	0.4	3.3	64		6	4.2	5.2	0
H	2	6.2	7.7	110					
	3	5.9	7.1	92					
	6	4.8	5.3	38					

In table 3 may be seen the 2- and 6-hour blood concentrations of isoniazid ("total" and "free") observed in a group of nontuberculous laboratory workers after ingestion of a dose of 10 mg. per kg. per day.

The results of investigation of the serum concentrations of isoniazid, "total" and "free," and PAS at the second, third, and sixth hours after drug administration to two groups of tuberculous patients are presented in table 4. Both groups of subjects received 10 mg. per kg. per day of isoniazid.[4] One group received 12 gm. of PAS[5] in addition; the other group received 6 gm. of PAS.[6]

Discussion

The data shown in tables 1, 3, and 4 reflect the reliability of the method described when applied to clinical research. It is evident from table 1 that the drug-free background of human serum in this method is sufficiently low to prevent any appreciable interference with the actual serum determinations. The data in both table 1 and table 3 show the remarkably specific pattern of metabolism of these drugs.

When the method, as described, was applied to urine, suitable results were obtained in the case of isoniazid, "total" and "free," but very high optical densities were observed in the case of PAS. Fortunately, the high concentrations of this drug which occur in the urine necessitate a 20:1 dilution to bring them within the range of the method, and this dilution results in suitably low optical densities for drug-free urine.

It has been found useful in the routine application of this method to employ a standard solution of these compounds in pooled, drug-free serum. When this is done it is necessary merely to solve the Bouger-Beer Law equation for the concentration of the drug in the specimens, since the dilution factors are eliminated. Moreover, this practice will ensure that any change in the chemical composition of the reagents will affect equally both the recovery of the standard and the recovery of the drug from the specimens, thus providing an automatic correction for this possibility in the calculation of the concentration. Finally, the routine employment of a standard solution will keep the technician informed of the status of his reagents and indicate when fresh reagents should be prepared.

The concentration of the standard should approximate the middle of the range of concentrations anticipated in the specimens, viz., for a two-hour specimen from a person receiving 10 mg. per kg. per day a standard with a concentration of about 3γ per ml. of serum should be suitable, while for the six-hour specimen from this same patient a standard having a concentration of 1γ per ml. should be employed.

The clinical application of the technique to urine, and the effects, if any, of pyrazinamide and streptomycin on the method are currently under investigation and will be the subject of a later report.

[4] Generously supplied as Nydrazid by E. R. Squibb and Sons.
[5] Generously supplied as Parasal by Panray, Inc.
[6] Generously supplied as Pascorbic by Hellwig, Inc.

Summary

A method is described for the quantitative determination of isoniazid, "free" and "total," and of *p*-aminosalicylic acid in body fluids. Data are presented to demonstrate the suitability of the method for routine clinical analyses or clinical research. Any clinical laboratory equipped with a spectrophotometer which can measure optical density at 365 millimicrons should be able to employ the method described without undue expenditure since very little additional special equipment is required. Two technicians can analyze 14 specimens daily, in addition to the blank and standard required.

Sumario

La Determinación Cuantitativa de la Isoniacida y del Acido Para-Aminosalicílico en los Humores Orgánicos

Descríbese un método para la determinación cuantitativa de la isoniacida, "libre" y "total," y del ácido para-aminosalicílico (PAS) en los humores orgánicos. Preséntanse datos para demostrar la adaptabilidad del método para los análisis clínicos corrientes o la investigación clínica. Todo laboratorio clínico provisto de un espectrofotómetro capaz de medir la densidad óptica a 365 milimicras debe poder emplear, sin mayor gasto, el método descrito, dado que requiere muy poco instrumental más. Dos técnicos pueden analizar 14 ejamplares al día, además del impreso y del patrón requeridos.

Resume

Détermination quantitative de l'isoniazide et de l'acide para-aminosalicylique dans les liquides organiques

Description d'une méthode de détermination de l'isoniazide, "libre" et "totale", et de l'acide para-aminosalicylique (PAS) dans les liquides organiques. Présentation de données démontrant que cette méthode est appropriée aux analyses et aux recherches clinques habituelles. Tout laboratoire clinique comportant appareillage un spectrophotomètre pouvant mesurer une densité optique à 365 millimicrons devrait pouvoir employer la méthode décrite sans dépense exagérée, car l' appariellage spécial supplémentaire qu'elle réclame est très réduit. Deux techniciens peuvent analyser 14 spécimens par jour, en plus des analyses simples et standard requises.

Acknowledgment

The writers gratefully acknowledge the assistance of Dr. Arthur Hellwig of Chicago who generously donated all of the *p*-aminosalicylic acid compounds used in the standardization of this method. They also wish to acknowledge their indebtedness to Dr. Hettie Hughes of Cincinnati, who generously supplied them with the isoniazid metabolites, except the acetyl derivative, used in this work.

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Case Reports

TWO CASES OF METHEMOGLOBINEMIA AND ACUTE HEMOLYTIC ANEMIA WITH DEATH FOLLOWING THE INGESTION OF A SOLUTION OF PARA-AMINOSALICYLIC ACID[1]

FRANCIS X. CLAPS

(Received for publication April 25, 1957)

Introduction

Since the institution of para-aminosalicylic acid (PAS) as a therapeutic agent in the treatment of tuberculosis in 1944, numerous reports have appeared regarding its toxicity. Anorexia, nausea, vomiting, and diarrhea may be noted as early symptoms and appear to be related to the tolerance of the patient to the drug (1). Itching, dermatitis, stomatitis, fever, enlargement of the thyroid, hepatitis with jaundice, and other hypersensitivity reactions have also been described (2–6).

The purpose of this paper is to describe two fatal cases of methemoglobinemia and acute hemolytic anemia following the ingestion of a solution of PAS.

Case 1. A 35-year-old white female, a known alcoholic for many years, was admitted to Bellevue Hospital on July 3, 1954. Four months prior to admission she was at another hospital with fever and pleural effusion of straw-colored fluid on the right. Cultures of the fluid and of gastric washings were negative for tubercle bacilli. The patient was discharged after three weeks on no specific therapy.

Six weeks before admission to Bellevue Hospital the patient had developed weakness, inability to walk, and mental confusion. On admission she appeared chronically ill. The rectal temperature was 98°F., the pulse rate 90 per minute, and the respiratory rate 16 per minute. The blood pressure in the right arm was 100 systolic, 60 diastolic in mm. of mercury. The only positive findings were calf muscle wasting and absent knee and ankle jerks, bilaterally. Due to the patient's mental condition, the remainder of the neurologic examination was unsatisfac tory.

Laboratory studies on admission revealed the following: The specific gravity of the urine was 1,017; the albumin test was negative and the sugar, negative. The blood hemoglobin was 9 gm. per 100 ml. and the erythrocyte count, 3,600 per cu. mm. The hematocrit was 32 mm. A serologic test for syphilis (Mazzini) was negative. The total plasma protein was 7.7 gm. per 100 ml. with albumin, 4 gm. per 100 ml. and globulin, 3.7 gm. per 100 ml. A cephalin flocculation test was negative; alkaline phosphatase, 6.0. The total cholesterol was 155; esters, 70; andl nonprotein nitrogen, 24 mg. per 100 ml. A roentgenogram of the chest on the tenth hospita day revealed elevation of the left leaf of the diaphragm, obliteration of the costrophrenic sinus on the right, and nodular infiltration in the right midlung field with strands extending to the right hilum. Compression and anterior wedging of the body of the tenth thoracic vertebrae were also noted. Intracutaneous injection of first-strength PPD gave a positive reaction.

The patient ran a febrile course, with the rectal temperature reaching 101°F. She refused to eat and required intermittent gavage. Cultures of the gastric washing were positive for tubercle bacilli.

On August 3, 1954, streptomycin, 1.0 gm. daily, and sodium para-aminosalicylic acid

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(PAS), 5 gm. three times daily, were started. On August 6 the patient received PAS from a stock solution bottle that had been standing in the medicine cabinet for an unknown period of time. It was black-brown in color.

Within two hours after the patient received the drug by gastric tube she developed nausea, vomiting, and diarrhea. The temperature rose to 104.8°F., the blood pressure fell to 76 systolic, 56 diastolic in mm. of mercury, and the regular but weak pulse rose to 116 per minute. The skin appeared cold, clammy, and brown in color. The serum was chocolate in color. The hematocrit fell to 19 mm. The output of urine dropped sharply. On examination it appeared black-brown in color and contained 2-plus albumin. Microscopic examination failed to reveal erythrocytes.

The patient was given oxygen by mask, arterenol intravenously, and cortisone; but she failed to respond. Just prior to death she appeared icteric, the nonprotein nitrogen rose to 60 mg. per 100 ml., the carbon dioxide, 5 mEq per L. She died twenty-one hours after ingesting the drug. Post-mortem examination was not performed.

Subsequent analysis of blood taken prior to the patient's death revealed methemoglobin. Chemical analysis of the urine revealed PAS, meta-aminophenol, salicylic acid, and a tarry black phenolic type of material which was otherwise unidentified.

Case 2. A 17-year-old Puerto Rican female was admitted to another city hospital on May 27, 1954, with polyarthritis and a generalized maculopapular erythematous rash. Positive physical findings were enlargement of the liver to 2 cm. below the costal margin and moderate generalized lymphadenopathy. Because of unusual mental behavior, she was transferred to Bellevue Hospital on June 28, 1954.

On admission the temperature was 98.6°F., the pulse rate, 90 per minute; respirations, 20 per minute; and the blood pressure was 130 systolic and 85 diastolic in mm. of mercury. Positive findings were: multiple, small deeply pigmented lesions, some crusted, located over the entire body; enlarged "shotty" axillary and inguinal lymph nodes; and a palpable liver 2 fingerbreadths below the right costal margin.

Laboratory studies on admission revealed the following: The specific gravity of the urine was 1,029; the albumin test was negative and the sugar, negative. The blood hemoglobin was 13 gm. per 100 ml. The erythrocyte sedimentation rate was 49 mm. per hour (Wintrobe corrected); the leukocyte count, 7,600 per cu. mm. A serologic test for syphilis (Mazzini) was negative. Nonprotein nitrogen was 35; total protein, 9.3; albumin 5.1; globulin, 4.2. A cephalin flocculation test was negative. Alkaline phosphatase determination was 2.6; total cholesterol, 234; and esters, 150. Roentgenographic examinations of the chest on numerous occasions were reported as normal. A biopsy of a skin lesion at Bellevue Hospital was reported as showing a granulomatous lesion of undetermined etiology in skin, probably tuberculosis. Cultures of gastric washings were repeatedly negative for acid-fast bacilli.

The patient was afebrile throughout her stay. Isoniazid (100 mg. three times daily), streptomycin (1 gm. twice a week), and PAS (3 gm. three times daily), were continued. On August 3, 1954, isoniazid was discontinued in order to re-evaluate the patient's mental status; PAS was increased to 4 gm. three times daily.

On August 6, 1954, the patient received the drug by mouth from a stock solution bottle that had been standing in a cabinet on the ward for an unknown period of time; it was the same solution used in *Case 1*. Within two hours she developed nausea, vomiting, and diarrhea. The temperature rose to 104.4°F., and the ventricular rate rose to 160 per minute. She developed a brownish discoloration of the skin. The serum was chocolate in color. The urine was black. The specific gravity was 1.013 and contained 1-plus albumin. On microscopic examination no erythrocytes were seen. The patient was gavaged and given oxygen by mask. The hematocrit fell to 25 mm. within ten hours. Icterus appeared and the blood pressure slowly fell despite the use of arterenol intravenously. A transfusion of 500 ml. of whole blood was given. Although urinary output was maintained throughout the patient's course, she died twenty hours after receiving this particular preparation of PAS. Just prior to death the

blood nonprotein nitrogen was 43 mg. per 100 ml., carbon dioxide, 11 mEq per L. Chemical analysis of blood both ante and post mortem revealed methemoglobin. As in the previous case, the urine contained PAS, meta aminophenol, salicylic acid, and a tarry black phenolic type of material, otherwise unidentified.

Autopsy was performed by Dr. Dominick DeMaio of the Medical Examiner's Office of the City of New York. The only abnormalities noted grossly were moderate enlargement of the liver and spleen and edema of the lungs.

Microscopic examination of the kidney revealed striking abnormalities. The distal convoluted tubules and the collecting tubules were distended by casts, some of which were pale pink and amorphous, but most of which were dark brown and granular. Sloughing and loss of lining cells were observed in many of the distal tubules with and without casts. In a few areas there seemed to be early extrusion of cast material into the interstitium. The affected tubules were surrounded by an interstitial infiltrate made up of lymphocytes and occasional polymorphonuclear leukocytes.

The epithelial cells of the proximal convoluted tubules were slightly swollen and the lumina contained a granular pink precipitate. The glomeruli were relatively bloodless but otherwise unaltered.

Sections of the lung revealed fairly extensive edema; scattered throughout were numerous interstitial granulomata made up of loosely packed mononuclear cells, some of which had an epithelioid character and a peripheral border of lymphocytes.

Sections of the heart revealed a normal epicardium and endocardium. The muscle fibers were well preserved but somewhat separated, and in the interstitium a mild diffuse plasma cell and lymphocytic infiltrate were accompanied by occasional neutrophils.

Sections of the liver revealed a single ill-defined periportal granuloma resembling those seen in the lung. No other abnormalities were noted in the liver.

Autopsy findings were interpreted as representing disseminated tuberculosis, regressing under chemotherapy. The single striking lesion was the pigment nephrosis, the dark brown cast of material in all likelihood representing the products of erythrocyte lysis and abnormal hemoglobin.

On chemical examination of the liver, para-aminophenol and meta-aminophenol could not be detected as such. An oily tarry substance which gave the reactions of phenol was obtained and probably represented a degradation product of PAS or its break-down products.

A sample of the stock solution of PAS which was given to the patients prior to their deaths contained 25 per cent PAS and 75 per cent degraded product. Of the 75 per cent degraded material, 17 per cent was meta-aminophenol and 58 per cent was a tarry black phenolic type of material, which probably was the toxic material.

Discussion

In 1950 three cases of acute hemolytic anemia with hemoglobinuria were described in the French literature (7) in 3 children who received PAS prepared in a solution commercially and dispensed in ampoule form. One child developed acute hemolytic anemia and hemoglobinuria within one hour of receiving the drug while the others had been receiving the medication for fifteen days before becoming ill. Two of the children survived on symptomatic treatment. In 1951 Burkens (8) described a case of acute hemolytic anemia, methemoglobinuria, and methemoglobinemia in a 21-year-old male who had been receiving 12 gm. of PAS in tablet form for one month before symptoms developed. The urine of this patient reportedly was negative for phenol or coal tar derivatives. On discontinuing the drugs, symptoms subsided.

A review of the literature discloses that the majority of cases of severe toxic reactions to PAS occurred in patients who received stock solutions of the drug. Many observers have attributed these toxic reactions to the presence of meta-aminophenol which is present in varying amounts, depending on the method used in manufacturing the drug

(2). Recent studies fail to confirm these findings (9, 10). The precise agent responsible for toxic reactions to this drug has not been identified.

Aqueous solutions of PAS and its sodium salt have been shown to darken rapidly in color on standing at room temperature (9). The decarboxylation rates of these solutions have been studied (11), and it has been shown that the solutions decompose rapidly at elevated temperatures and low pH. Light and high concentrations of carbon dioxide augment their breakdown.

When solutions of PAS are to be used, it is best to prepare the solution at the time it is to be dispensed. The sodium salt would seem to be preferable as it is much more soluble in water. If it is necessary to prepare stock solutions, then the solution should be kept in a dark bottle tightly stoppered in a refrigerator. Even with these measures, it is perhaps better not to use the solution after five days.

Summary

Since para-aminosalicylic acid (PAS) was introduced as a therapeutic agent in the treatment of tuberculosis, numerous reports have appeared regarding its toxic reaction. The present report describes 2 cases of methemoglobinemia and acute hemolytic anemia following the ingestion of a solution of PAS. In both cases the patients had tuberculosis and had been treated with PAS. Following the ingestion of a stock solution of the drug that had been standing for quite a while and was black, the patients went into shock, became blue, and developed anuria. Both patients died within twenty-four hours, and analysis of the blood revealed the presence of methemoglobin. Urine examination revealed PAS, meta-aminophenol, salicylic acid, and a tarry black phenolic type of material otherwise unidentified. A sample of the stock solution which was given to the patients prior to their deaths contained 25 per cent of PAS and 75 per cent of degraded material. Of the 75 per cent of degraded material, 17 per cent was meta-aminophenol and 58 per cent a tarry black phenolic type of material, which was probably what produced the toxic reaction.

Sumario

Dos Casos de Metahemoglobinemia y de Anemia Hemolítica Aguda con Muerte Consecutiva a la Ingestión de una Solución de Acido Para-Aminosalicílico

Desde que se introdujo el ácido para-aminosalicílico (PAS) en el tratamiento de la tuberculosis, han aparecido numerosas comunicaciones acerca de su reacción tóxica. La comunicación actual describe dos casos de metahemoglobinemia y de anemia hemolítica aguda consecutivas a la ingestión de una solución de PAS. En ambos casos, los enfermos tenían tuberculosis y habían sido tratados con PAS. Después de la ingestión de una solución almacenada de la droga, que había estado preparada por bastante tiempo y tomado color negro, los enfermos cayeron en choque, se cianosaron y manifestaron anuria. Ambos enfermos fallecieron en término de veinticuatro horas y el hemánalisis reveló la presencia de metahemoglobina. El uranálisis reveló PAS, meta-aminofenol, ácido salicílico y una substancia embreada, negra, de tipo fenólico, que no pudo identificarse más. Una muestra de la solución almacenada que se administró a los enfermos antes del fallecimiento contenía 25 por ciento de PAS y 75 por ciento de materias degradadas. De este 75 por ciento, 17 por ciento era meta-aminofenol y 58 por ciento una substancia embreada, negra, de tipo fenólico, que fué probablemente la que produjo la reacción tóxica.

Resume

Deux cas de méthémoglobinémie et anémie hémolytique aiguë avec décès après ingestion d'une solution d'acide para-aminosalicylique

Depuis que l'acide para-aminosalicylique (PAS) fut introduit comme agent thérapeutique dans le traitement de la tuberculose, de nombreux rapports ont été publiés à l'égard

de sa réaction toxique. Le rapport actuel décrit deux cas de méthémoglobinémie et d'anémie hémolytique aiguë succédant à l'ingestion d'une solution de PAS. Dans les deux cas les patients étaient atteints de tuberculose et avaient été traités par le PAS. A la suite de l'ingestion d'une solution standard de la drogue qui avait été préparée depuis assez longtemps et qui avait une coloration noire, les malades présentèrent un état de choc, devinrent cyanosés et présentèrent de l'anurie. Ces deux patients moururent dans les vingt-quatre heures et l'analyse du sang révéla la présence de méthémoglobine. L'analyse de l'urine révéla la présence de PAS, de méta-aminophénol, d'acide salicylique et d'une matière de type phénolique d'un noir goudronneux non identifiée. Un échantillon de la solution standard administrée aux malades avant leur décès contenait 25% de PAS et 75% d'une substance décomposée. Sur ces 75% de substance décomposée, 17% étaient du méta-aminophénol et 58% une matière phénolique d'un noir goudronneux, celle-ci étant probablement à l'origine de la réaction toxique.

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(7) Paris med., 1950, *40*, 659 (abstract).

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(10) Mitchell, S. R., Schmidt, J., Copel, L., Schell, L.: Study of para-aminosalicylic acid intolerance in tuberculosis of lung, J. A. M. A., 1954, *156*, 124.

(11) Oberweger, K. H., Seymour, D. E., Simmonite, D.: Para-aminosalicylic acid: Analytical and pharmaceutical studies, Quart. J. Pharm. & Pharmacol., *1948*, 21, 292.

Notes

A STUDY OF AMINO ACID METABOLISM WITH URINE FROM TUBERCULOUS PATIENTS

Filter paper chromatography has become a generally useful tool for the detection of amino acids in body fluids.[1] Amino acids in urine can be an expression of normal as well as pathologic metabolism,[2, 3] The specific interest of the present writer was the study of amino acids in the urine of tuberculous patients, with a view to the possible influence of treatment with chemotherapy such as PAS. This paper describes only a preliminary study in order to find out whether it would be of interest to do research in this direction. The results are encouraging; in fact, they open up such vistas as to stimulate one to enlarge on it as much as possible in the future.

Method: After some consideration and trial, the one-dimensional method on Whatman paper No. 1 of chromatographic quality and methyl-ethyl-ketone, propionic acid was used with water in a volume ratio of 75:25:30 as eluent, which has a great resolving power and relatively fast migration. The descending method in one of the commercially manufactured chromatocabs was used. The filter paper strips were 4 inches wide and 22 inches long; the eluent period was twenty hours. The chromatogram can be air-dried in thirty minutes. An 0.2 per cent solution of ninhydrin in ethyl alcohol (95 per cent) was used as reagent. The method is described and discussed by Clayton and Strong[4] and was followed closely except that the descending method was used. The R_f values agreed closely with others reported in the literature.

Urine, 0.03 ml., or, preferably 0.06 ml., was applied to the initial location on top. It was found that the morning urine gave as reliable a result as the 24-hour collection. Usually in the present cases a late afternoon urine would show a somewhat different intensity.

The urine was not desalted for it was believed that, by desalting, particles of sometimes minute amounts of amino acids might adhere to the precipitate and get lost.[5] With the method used, the separation was quite satisfactory and no visible or significant interference by other components of the urine was observed, contrary to the opinion of some investigators. This was evidenced by the fact that control specimens with pure amino acids, single or in mixture, resulted in almost the same R_f value. For identification, the R_f value was used with simultaneous runs of pure amino acids beside the specimen for comparison.

The color rating in terms of *I* (very strong), *II* (strong), *III* (medium), *IV* (weak), *V* (very weak), *VI* (faint), *VII* (absent) corresponds in color intensity with the standards in mg. per 1,000 ml. of urine as follows:

	I	II	III	IV	V	VI	VII
Alanine	333	150	66	50	41	17	0
Arginine	333	150	80	58	33	7	0

[1] *a*. Block, R. J., Durrum, E. L., and Zweig, A.: A Manual of Paper Chromatography and Paper Electrophoresis, Academic Press, Inc., New York, 1955. *b*. McDonald, M: Ionography, The Year Book Publishers, Chicago, 1955. *c*. Rudolph, W.: Biochemie des Aminosäurestoffwechsels, Medizinischer Verlag Hans Huber, Bern, Switzerland. *d*. Cramer, F.: Papierchromatographie, ed. 3, Verlag Chemie, 1954. *e*. Martin, A. J. P.: Endeavour, 1947, *6*, 21.

[2] Spritzer, A.: Amino-aciduria and related clinical syndrome (manuscript).

[3] *a*. Dent, C. E.: Lancet, 1946, *2*, 637. *b*. Dent, C. E., and Rose, G. A.: Quart J. Med., 1951, *20*, 215.

[4] Clayton, R. A., and Strong, F. H.: Analyt. Chem., 1954, *26*, 1362.

[5] Mueller, G. C., Bowman, G., and Herranen, A.: Analyt. Chem., 1954, *26*, 1357.

	I	II	III	IV	V	VI	VII
Aspartic acid	333	167	83	41	17	6	0
Cystine	—	124	100	83	41	8	0
Glutamic acid	316	140	66	41	21	8	0
Glycine	92	66	41	15	8	6	0
Histidine	333	166	83	41	17	8	0
Leucine	125	83	41	21	8	2	0
Lysine	333	166	83	50	25	12	0
Methionine	333	166	83	41	30	17	?
Phenylalanine	323	166	83	41	25	8	0
Threonine	333	166	83	41	17	8	0
Tyrosine	167	108	66	27	17	8	0

This fact allows a semiquantitative estimation of the presence of all reported amino acids. Re-elution of the ninhydrine spots and quantitative determination of the amino acids with a spectrophotometer[6] was not attempted because the aim was to work out a simple routine laboratory method for use by any clinical laboratory.

Also, an "artificial" urine was made by using the approximate amount of the major components of urine in chemically pure form with and without the addition of amino acids in order to find out whether the salts, et cetera, in the solution would cause a shift of the R_f factor. The formula of this artificial urine per 1,000 ml. was: urea, 30 gm.; uric acid, 0.6 gm.; sodium chloride, 5 gm.; potassium sulfate, 3.0 gm.; potassium phosphate, 2.5 gm.; magnesium sulfate, 0.4 gm.; calcium nitrate, 0.7 gm. This formula, of course, is only an approach to the natural urine. Three-hundredths of a milliliter were used. The blank, of course, showed nothing. Amino acids, added singly and combined, showed up at the proper location and in the proper strength, corresponding to the six color standards.

The following twelve amino acids were identified in the order of their distance from the point of origin: cystine (45 mm.), lysine (63 mm.), histidine (72 mm.), arginine (74 mm.), aspartic acid (85 mm.), glycine (87 mm.), threonine (105 mm.), glutamic acid (115 mm.), alanine (118 mm.), tyrosine (156 mm.), methionine (180 mm.), leucine (206 mm.), proline and valine were observed only in a few cases and are not reported here.

Ninety cases of hospitalized patients were investigated and are reported. The morning urine before breakfast was used in all cases. Another specimen of the patients who received PAS was used, taken about one hour after taking PAS. The urine needed no preservative since it was processed the same day.

Of the 90 cases investigated, 35 patients were under treatment with PAS. Some of these patients showed a distinct change of pattern within an hour after taking PAS. This drug shows up on the chromatogram as a faint but clearly visible yellow spot just below leucine (descending method). It could be followed in the urine up to four hours after administration in most cases, in a few even longer. The typical change of pattern occurred within one to two hours. However, it was not possible to make complete surveys over longer periods of time. Therefore, it is impossible to tell whether the noted effects of PAS are temporary or long lasting.

After ingesting PAS, the changes in the amino acid pattern are: no change, increase of the same amino acid above the pre-PAS level, decrease below the pre-level, total disappearance, appearance of other amino acids not previously observed in the same patient. These findings are reported in the following table:

[6] Block, R. J., and Bolling, D.: The Amino Acid Composition of Proteins and Foods, Charles C Thomas, Springfield, Illinois, 1945.

Amino Acid	No Change	Increase	Decrease	Disappearance	Appearance Number	Total Number of Cases
Alanine	22.5	22.5	40.5	14.5	1	22
Arginine	9.1	0	18.2	72.7	2	11
Aspartic acid	33.0	26.4	30.7	9.9	3	30
Cystine	40.5	14.5	18.0	27.0	3	22
Glutamic acid	46.0	25.0	21.0	8.0	4	24
Glycine	35.0	20.0	30.0	15.0	3	20
Histidine	48.2	24.1	17.3	10.4	1	29
Leucine	41.2	0	10.8	48.0	3	17
Lysine	54.3	5.8	34.1	5.8	0	35
Methionine	16.5	30.7	46.2	6.6	0	30
Phenylalanine	50.0	15.0	25.0	10.0	0	20
Threonine	45.8	4.2	41.6	8.4	0	24
Tyrosine	41.4	20.6	34.5	3.5	0	29

The depressing effect of PAS is most noticeable in the following amino acids: arginine, glutamic acid, glycine, leucine, phenylalanine—depressing effect; lysine—slightly raised values; methionine and threonine—raised values. Apparently these shifts are not due to any dietary influence, for Eckhart concludes that the increase of protein in test diets will raise only slightly the urinary excretion. Hier[7] mentions that there is a slightly reduced amino acid excretion in low protein diets, but there was no direct correlation between protein intake and amino acid excretion. This is important, for it may be assumed that the findings here reported are not the result of diets. It has been reported by Pierce that a diet deficient in one essential amino acid may increase the excretion of all amino acids. In the present investigation (and this seems to be significant) there was the problem of reduced excretion more often than that of increased excretion. Further information on increased or reduced excretion will be found in references[8–19].

In summary: In studies of amino acids in the urine of tuberculous patients receiving PAS, values for arginine, glycine, leucine, and phenylalanine were found to be significantly low; histidine and tyrosine were moderately low; aspartic acid and cystine were close to normal; lysine was normal or slightly raised; methionine and threonine showed raised values; PAS showed a markedly depressing influence, especially between one and two hours after intake.

[7] Hier, S.: Tr. New York Acad. Sc., 1947, *2*, 10.
[8] Perry, J. W., *et al.*: M. J. Australia, 1954, *41*, 812.
[9] Jaraschka, R., and Kresbach, H.: Wien. klin. Wchnschr., 1954, *66*, 762.
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[18] Lehrbuch der Speziellen Pathologischen Physiologie, Herausgegeben von Ludwig Heilmeyer, Gustav Fischer Verlag, Stuttgart, 1955.
[19] Outline of the Amino Acids and Protein, edited by Melville Sahyun, Reinhold Publishing Corporation, New York, 1948.

In the evaluation of all findings, the functional and pathologic role of each amino acid needs to be considered separately.

A research program on a broad basis should be undertaken following selected cases over longer periods of time in order to demonstrate the shift due to improvement or worsening of the disease, as well as the influence of chemotherapy. In this way it is possible that renal and hepatic damage can be discovered long before a critical stage is reached. The presence in various strengths of certain amino acids in the urine might then become an additional test along routine laboratory lines for the progress in the treatment of the tuberculous patient.

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ATYPICAL HISTOLOGIC ASPECTS OF PULMONARY TUBERCULOSIS AS RELATED TO ATTENUATION OR LOSS OF PATHOGENICITY OF ISONIAZID-RESISTANT STRAINS OF M. TUBERCULOSIS

It is well known that strains of tubercle bacilli under the influence of prolonged chemotherapy become increasingly resistant to the drug employed after a period of time which varies according to the clinical case and the therapeutic regimen. It is also known that, when the therapeutic regimen consists of streptomycin and/or PAS, the isolation of drug-resistant strains is, as a rule, followed by worsening and relapse. However, isolation of isoniazid-resistant strains is not followed, in the same proportion, by deterioration of the clinical and roentgenographic picture. This fact is paralleled by alteration of the biologic characteristics of the isolated strains, which tend to lose their catalase activity and pathogenicity for guinea pigs and other laboratory animals.

As has been seen frequently, even in cases of generalized visceral tuberculosis of guinea pigs, attenuation or loss of pathogenicity of the challenging isoniazid-resistant strains may be evidenced by the regressive nature and the absence or slight degree of caseation of the lesions three months after inoculation of massive doses (1 mg. wet weight).

Despite this peculiar action on tubercle bacilli, isoniazid seems to modify the histopathologic picture of tuberculosis in humans in a particular way.[1, 2] At least, some atypical morphologic features are more commonly found after the use of isoniazid.[3, 4] Classically, epithelioid and giant cells are found in the tuberculous granulomata or on the periphery of caseous areas. Since isoniazid was brought into use, these cellular elements have not infrequently been found in atypical situations, away from any tubercle, loose within alveolar spaces, "out of context," as Denst said.[1] Even in the interior of bronchioles one may observe giant cells, sometimes coated by mucus.[2] At this point it may properly be asked if there is any relationship between the attenuation or loss of pathogenicity of isoniazid-resistant strains and the atypical morphologic aspects probably connected with the action of this drug. Some facts suggest that this may well be the case.

The finding of giant cells "out of context," within alveolar spaces, was registered in experimental but not in human tuberculosis before the antimicrobial era. In 1933, Leuret and Caussimon,[5] studying the lung tissue reaction of rabbits to the intravenous or intratracheal inoculation of inert particulate matter, dead virulent bacilli, BCG, or living virulent bacilli, verified that everything happened as if the lung tried to eliminate the inert, toxic, or virulent particles from the septa to the alveoli and the bronchial tree. The reaction pattern was always the same, but the ease of alveolar elimination was inversely proportional to the toxicity or virulence of the particles. The reaction was fundamentally based upon the macrophages as such or as transformed into epithelioid and giant cells, invading extensively the lung tissue or tending to form circumscribed lesions. Atypical and more or less evanescent granulomata were seen especially in connection with the injection of inert particulate matter, dead virulent bacilli, or BCG. On the con-

[1] Denst, J.: Am. Rev. Tuberc., 1953, *68*, 144.

[2] Poppe de Figueiredo, F., and De Paola: Am. Rev. Tuberc., 1955, *71*, 186.

[3] Canetti, G.: Symposium on Human Tuberculosis, Ciba Foundation, London, 1955, p. 283.

[4] Canetti, G.: Presse méd., 1956, *64*, 129.

[5] Leuret, E., and Caussimon, J.: Les Réactions du Tissu Pulmonaire dans la Tuberculose, Masson et Cie., Paris, 1933.

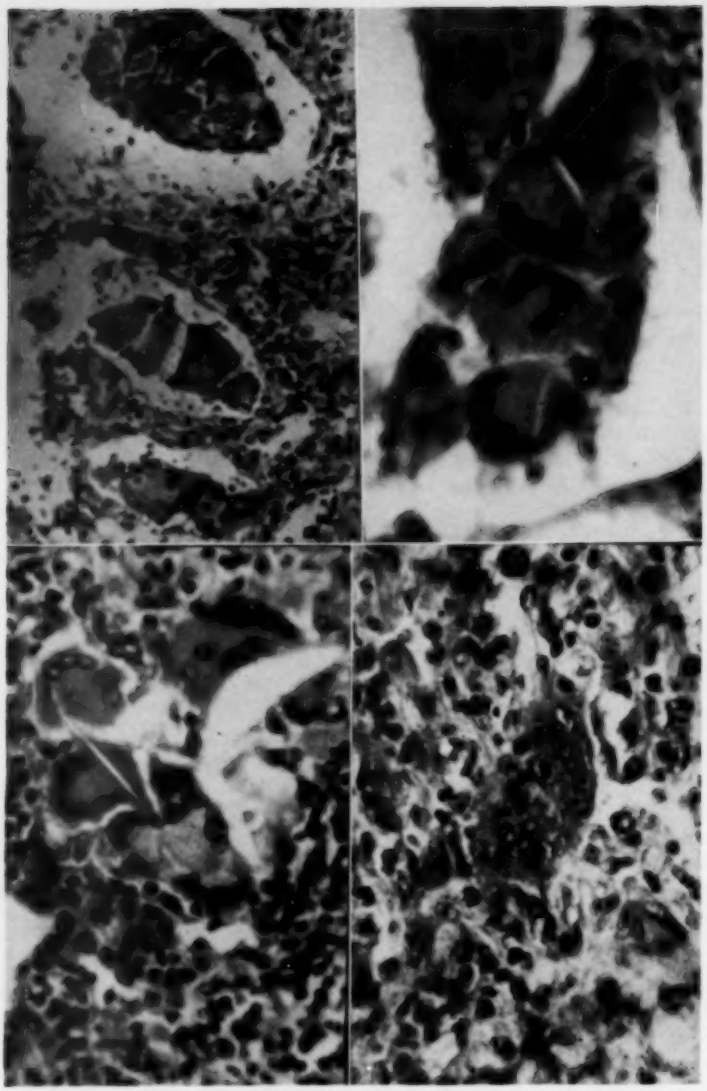

FIG. 1 (Upper left). Atypical giant cells within air spaces. The patient was under long-term chemotherapy. Note calcified Schaumann bodies in the cellular mass at the top, crystal slits in the middle giant cell, and a small asteroid body in the inferior giant cell.

FIGS. 2 and 3 (Upper right and lower left). Giant cells within air spaces. Several of them have few nuclei. Note also crystal slits. The patients were under long-term chemotherapy.

FIG. 4 (Lower right). Compact epithelioid and giant cell pneumonia in a patient under prolonged chemotherapy.

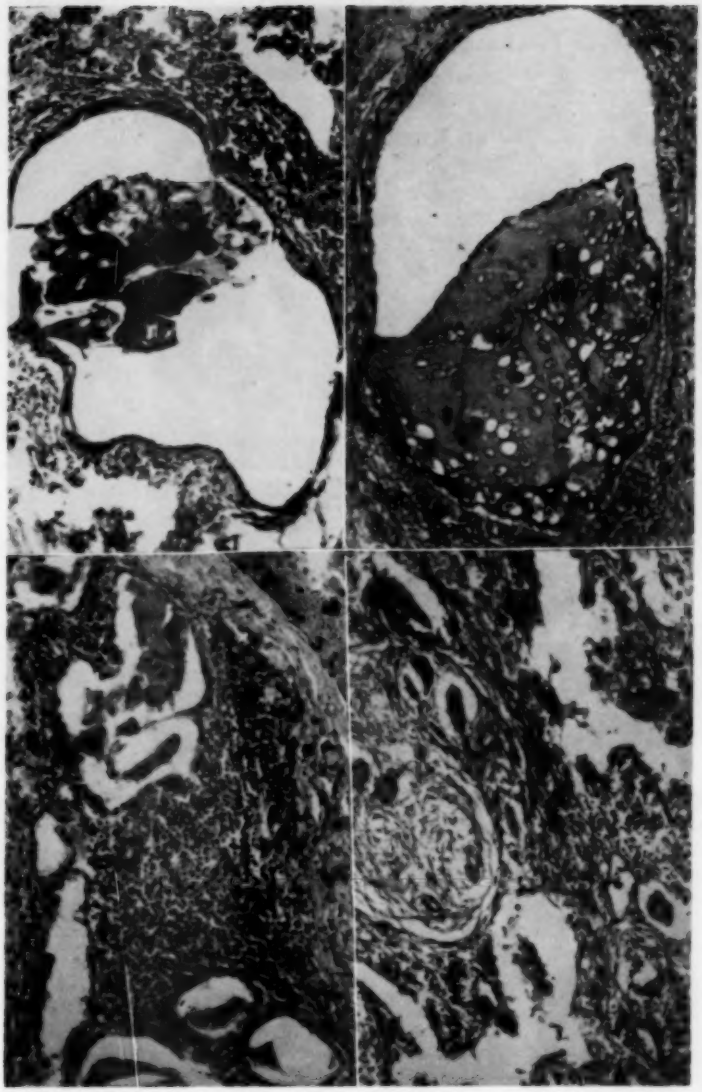

FIG. 5 (Upper left). Giant cells forming a pedunculated mass within a bronchiole. The patient was treated with antimicrobial drugs.

FIG. 6 (Upper right). Giant cells, coated by mucus, within a bronchiole. The patient was under prolonged chemotherapy.

FIG. 7 (Lower left). Atypical giant cells within bronchioles and air spaces. The patient was under long-term chemotherapy.

FIG. 8 (Lower right). Granulomata showing atypical giant cells, some of which are competely loose. The patient was under prolonged chemotherapy.

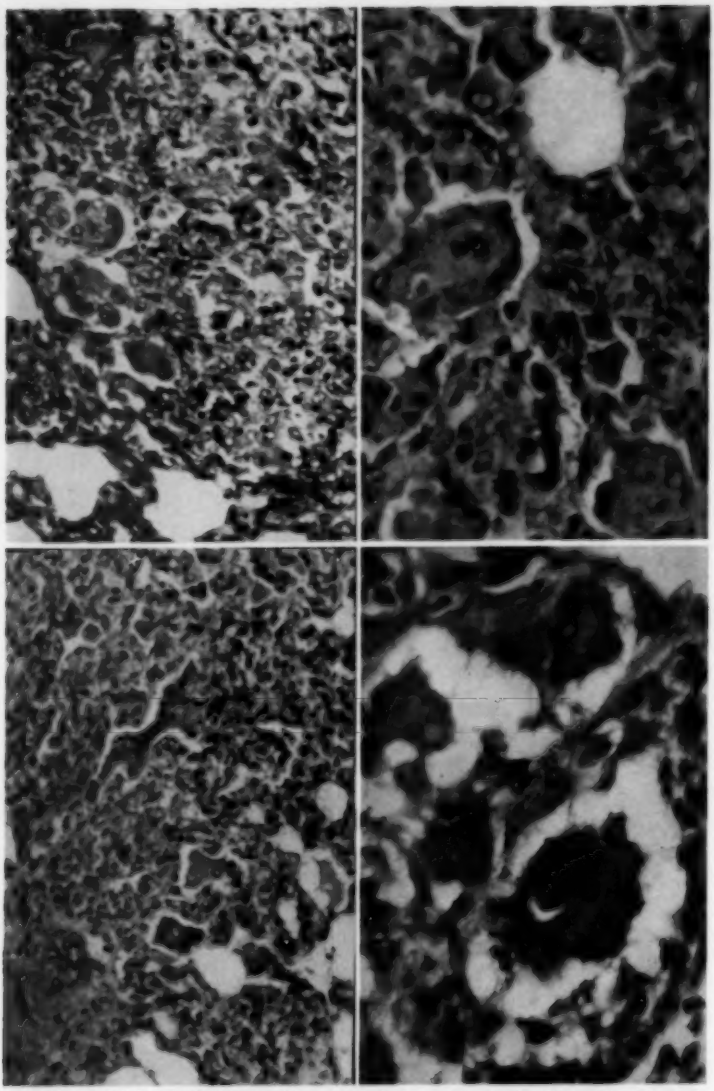

FIG. 9 (Upper left). Epithelioid cell pneumonia in a guinea pig. Several nucleated giant cells are seen within alveolar spaces.

FIG. 10 (Upper right). Epithelioid cell pneumonia in a guinea pig inoculated with 1 mg. of the strain *SM 3* (resistant to 50γ of isoniazid per ml. of medium, catalase-negative, and of low pathogenicity). High power magnification shows giant cells within alveolar spaces.

FIG. 11 (Lower left). Epithelioid pneumonia in a guinea pig inoculated with 1 mg. of the strain *SM 3*. Several small and atypical giant cells may be seen within alveolar spaces.

FIG. 12 (Lower right). Epithelioid cell pneumonia in a guinea pig inoculated with 1 mg. of the strain *SM 244* (resistant to 5γ of isoniazid per ml. of medium, catalase-negative, and of low pathogenicity). High power magnification to show giant cells becoming loose within alveolar spaces.

trary, typical and chronic granulomata would mean a more or less prolonged fixation of the virulent foreign bodies and their chemical fractions, due to the impossibility of their complete destruction and elimination with final *restitutio ad integrum*. So, teleologically speaking, progressive caseous-exudative lesions could be interpreted as a total failure of the cellular reaction in trying to destroy and eliminate the virulent particles by alveolar and bronchial routes.

The presence of small giant cells with 3 to 10 nuclei within alveolar spaces had already been registered by Coulaud in his classic work on the experimental infection with BCG.[6] Leuret and Caussimon also verified that following the inoculation of normal rabbits with virulent bacilli, alveolar elimination of epithelioid and giant cells might eventually be observed as an expression of resolution of a compact cellular pneumonia. In this case, other factors, such as the infecting dose and the natural resistance of the animal, must be invoked. In the allergic animal such elimination probably does not occur or is more difficult. Experimental production of compact epithelioid cell pneumonia—a condition in which alveoli become filled with solid plugs of epithelioid and giant cells—was still achieved by Rich and McCordock,[7] introducing a moderate number of virulent bacilli into the alveoli of animals with low or absent hypersensitivity or a small number of virulent bacilli into the alveoli of the hypersensitive body.

According to Rich, in the human being the same conditions doubtless determine the occurrence of this type of lesion, the bacilli reaching the alveoli from a cavity or from a caseous lymph node with few bacilli that have perforated a bronchus. If the perforated bronchus is a large one, the process may assume all clinical and roentgenographic features of the so-called epituberculosis. Such an epithelioid and giant cell pneumonia clears gradually in serial roentgenograms. Its relative infrequency and benign character, as well as the allergic state, could explain the absence of observations on giant cells in atypical situations within alveoli and bronchioles before the routine use of pulmonary resection and antimicrobial drugs in the treatment of tuberculosis. As a matter of fact, as far as is known, the finding of loose giant cells within alveoli and bronchioles had never been reported in tuberculous patients who died of tuberculosis or other causes before the antimicrobial era.

In these last years the present writers have frequently seen compact epithelioid cell pneumonia in guinea pigs inoculated with massive doses (1 mg. wet weight) of isoniazid-resistant strains isolated from patients under prolonged chemotherapy. The consolidation may assume a more or less circumscribed and granulomatous appearance, but very commonly it extends over a large area of the lung. In these pneumonic patches giant cells may be seen "out of context," loose within alveoli, and it is interesting to note that such cells are small, as a rule, and include a few nuclei.

Canetti[3] described this type of lesion in human tuberculosis treated by antimicrobial drugs as a surprising picture of epithelioid cell pneumonia or giant cell pneumonia, which is sometimes widespread and, in extreme cases, resembles a tumor. The phenomenon could perhaps be explained by easy destruction and elimination of dead bacilli under the influence of antimicrobial drugs, as may be seen after the experimental inoculation of bacilli killed by physical means. However, the situation of the normal animal inoculated with dead bacilli and of the allergic patient treated with antimicrobial drugs is not the same. Moreover, such atypical pictures are not registered in the literature concerned with the action of streptomycin on human tuberculosis.[2] They have been described after

[6] Coulaud, E.: Ann. Inst. Pasteur, 1927, *41*, 289.

[7] Rich, A. R.: The Pathogenesis of Tuberculosis, Charles C Thomas, Springfield, Illinois, 1951, pp. 842–843.

the use of isoniazid, which, being almost always the only factor of attenuation or loss of pathogenicity of the tubercle bacilli, exerts at the same time an inhibitory effect on the allergic sensitivity of the body.[8] This fact suggests that the easy elimination of giant cells by alveolar and bronchiolar routes is connected with attenuation or loss of pathogenicity of isoniazid-resistant strains per se or through the effect of isoniazid on allergy.

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[8] Choremis, C., *et al.*: Am. Rev. Tubere., 1956, 74 (Supplement, p. 197).

[9] The writers wish to express their sincere appreciation for the collaboration of Dr. Milton Fontes Magarao.

STUDIES OF CONJUGATED PAS AND ASCORBIC ACID AND OTHER FORMS OF PARA-AMINOSALICYLIC ACID[1]

I. Patient Tolerance

Para-aminosalicylic acid (PAS) continues to maintain an essential place in the therapy of tuberculosis. Despite its disadvantages, including intolerance, relatively large dosage, and expense, its qualities as a companion to one of the more potent antimicrobials have been sufficient to ensure its continued large-scale usage.

In addition to delaying the emergence of tubercle bacilli resistant to either of the two major drugs, it permits their long-term *successive* rather than *simultaneous* use. The total period of effective coverage is thereby extended—an important factor in the patient who received late surgery or one who has to live with persistent cavity. Recently Morse and co-workers[2] have presented evidence that PAS administered with isoniazid may increase the blood concentration of active unconjugated isoniazid by consuming part of the available acetylating capacity of the body. This would seem to be an additional recommendation for the use of PAS with isoniazid.

In the search for a more desirable and effective form of PAS, Scheel[3] has synthesized a conjugated PAS-ascorbate (Pascorbic®). The present study is concerned with patient tolerance of PAS-ascorbate in contrast to acid PAS and sodium PAS.[4] Serum concentrations attained with PAS-ascorbate and certain other forms of PAS will be reported in the second and third papers of this series. No attempt is made at this time to evaluate therapeutic and bacterial resistance aspects of PAS-ascorbate, or to estimate the importance of this drug in preventing the emergence of tubercle bacilli resistant to companion drugs.

Chemotherapy with PAS is frequently limited because of gastrointestinal symptoms.[5, 6] All too often these side-reactions deprive the patient of the benefits of this drug in long-term antituberculosis treatment. The symptoms consist mainly of nausea, vomiting, diarrhea, and abdominal cramps. The incidence and severity of symptoms vary greatly from patient to patient and from time to time in the same patient. Evaluation of symptoms associated with a new form of PAS is important. Therefore, the following study was carefully devised to compare patient tolerance of PAS-ascorbate with that of sodium PAS and the acid form of PAS.

Twenty-two adult patients were studied. The only restricting criteria were that the patient would remain in the hospital without surgery for six months and that he would cooperate in permitting multiple venipunctures. Most of the patients were receiving various forms and dosages of PAS prior to the study, but only a few had previous PAS therapy for more than three weeks. Fifteen of the 22 patients completed the study. Three patients left the hospital against medical advice. Two patients were discharged. One patient developed a cerebrovascular episode during the study and another patient experienced an increase of pulmonary tuberculosis causing difficulty in accurate interpretation of his symptoms.

[1] This investigation was supported in part by a grant from the Ohio Tuberculosis and Health Association.

[2] MORSE, W. C., *et al.*: Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 283.

[3] SCHEEL, L. D.: Personal communication.

[4] The several forms of PAS used in this study were generously supplied by Arthur P. Hellwig, of Hellwig, Inc., Chicago, Illinois.

[5] MADIGAN, D. G.: Lancet, 1950, *1*, 239.

[6] BOGER, W. P., AND PITTS, F. W.: Am. Rev. Tuberc., 1950, *62*, 610.

The patients were informed that three new, different, and purified forms of PAS would be given to them in rotation during the study. The drug would be administered to them daily in four doses. Each patient was instructed and observed by the nursing staff to take a dose at each mealtime and at 9 p.m. with or without food. The study was a "double blind" one in that each form of PAS supplied to this hospital was identified only by a number. The identity of the variously numbered forms was unknown to the hospital staff or patient. The three forms of PAS were produced from the same pure lot of acid PAS. All drugs were supplied by the manufacturer in tablet form, each tablet containing the equivalent of 0.5 gm. of acid PAS. The tablets were of identical appearance and were supplied in packets of six tablets.

Assignment of patients to different forms of drug was done by random choice. Following either a month of therapy or less if the drug was not tolerated, the patients were rotated to a different form of PAS, again by random choice. This allowed approximately one-third of the patients to be on each form of the drug at one time.

To simplify and to increase the validity of criteria for intolerance, such vague symptoms as anorexia and nausea were omitted from consideration. Intolerance in this study was therefore defined in terms of diarrhea, vomiting, or refusal to take the drug. The use of diarrhea as a symptom was based on an increase in the number of daily stools to an annoying level and a change in the character of the stool. When a patient developed symptoms of vomiting, diarrhea, or refused to take the drug, administration was discontinued for 48 hours. Then a full dose of the same drug was re-started. The 48-hour rest period was used to help exclude other causes of the symptoms and served to indicate the relationship between the administration of the drug and the symptoms. If symptoms recurred after resumption of the drug in full doses, the drug was discontinued for an additional 48 hours. Following this rest period *another* form of PAS was administered.

TABLE 1

TOLERANCE TO PAS ADMINISTRATION

	Acid PAS: 30 Days	PAS-Ascorbate: 30 Days	Sodium PAS: 30 Days
LA	−	−	−
HA	+(r)	+(r)	+
VB	−	−	+(r)
NB	−	−	+
GG	+	+	+
JG	+(r)	+	+
BH	+	+	+
HH	−	−	+
CH	−	−	+(r)
GL	−	−	−
LM	+	+	+
JM	−	−	−
MP	+	−	+
EW	+	+(r)	+(r)
SY	−	−	−
Tolerated	7	6	11
Not tolerated	8	9	4

+ = tolerated; − = not tolerated; +(r) means that the drug was tolerated when resumed following a period of intolerance succeeded by a rest period.

The results of this study are seen in table 1, in which is shown the number of patients who could tolerate full doses of each drug for a total of one month. It is seen that almost twice as many patients tolerated the sodium salt of PAS as tolerated the free acid or PAS-ascorbate forms. The latter 2 produced approximately the same degree of unfavorable symptomatic response. It was noted that some patients could resume the original form of the drug successfully for a longer period following the first 48-hour rest period (*see* +(r) in table 1).

In several instances (patients *HA*, *GG*, *JG*, *BH*, *IM*, and *EW*), all forms of PAS were tolerated for a month each, although some needed a 48-hour rest period (*see* +(r) in table 1). Of the three drugs, only sodium PAS was tolerated by patients *VB*, *NB*, *HH*, and *CH*. Certain patients, *LA*, *GL*, *JM*, and *SY*, could tolerate none of the three forms for a full thirty days. There was no patient intolerant to sodium PAS who could tolerate full doses of either of the other forms of PAS.

Thirteen of the 15 patients were additionally given PAS-ascorbate in half doses (6 gm. daily) to observe tolerance and serum concentrations. Only 2 of these patients developed any symptoms. In one case a brief rest from drug therapy was required, but the patient was later re-started on half doses of PAS-ascorbate and continued on this drug without difficulty.

The findings of this study of patient tolerance, even though the numbers were small, serve to confirm clinical impressions. The present writers have been impressed with the irregularity of response of patients to one or another form of PAS at different times—being intolerant at first and tolerant at a later date after a rest period or when another form had been used during the intervening period.

In summary: It is apparent from this study that the majority of patients can tolerate sodium PAS in full dosages, but many cannot tolerate either acid PAS or PAS-ascorbate in full dosage. Some patients tolerate any of these three forms of the drug without manifesting important symptoms. Likewise, some patients cannot tolerate full dosages of any of these forms of PAS. Those patients who do not tolerate a full dose of acid PAS or PAS-ascorbate can usually tolerate half doses of PAS-ascorbate. This study suggests that undesirable reactions related to PAS-ascorbate are of the same magnitude as those associated with acid PAS.

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February 15, 1957

STUDIES OF CONJUGATED PAS AND ASCORBIC ACID AND OTHER FORMS OF PARA-AMINOSALICYLIC ACID[1]

II. Comparison of Twenty-Four-Hour Blood Serum Concentrations

This is the second report of studies[2] of a newly synthesized form of PAS known as conjugated PAS-ascorbate (Pascorbic®). The present report deals with blood serum concentrations attained with administration of PAS-ascorbate and certain other forms of PAS.

In order to compare serum concentrations throughout the 24-hour period, and especially to observe the maintenance of overnight concentrations, a number of forms of PAS were administered under standard conditions.

Some of the compounds studied have been the subject of reports by other investigators.[3–6] Claims of higher serum concentrations have been made by the investigators and, in some instances, in advertisements.

Three groups of adult male tuberculous patients who tolerated full doses of sodium PAS were selected. The particular form of PAS to be studied was administered in four equal doses at 8 a.m., 12 noon, 5 p.m., and 9 p.m., the standard medication hours for PAS at this hospital. The total 24-hour dosage in each instance equalled 12 gm. of free PAS. On the third day of administration, standard identical diets were served so that absorption of the drug would be equally affected by dietary intake throughout the study. Fluid intake was not controlled.

Blood was obtained from arm veins at the following times during the third day of administration of each drug: 8 a.m., 10 a.m., 12 noon, 2 p.m., 5 p.m., 7 p.m., 11 p.m., or 12 midnight, and 8 a.m. (eight determinations in 24 hours for each patient and each drug studied). Serum concentrations of PAS were determined by the Marshall method (modified). Because of the effect of light on the chemical reactions involved in this procedure,[7] all determinations were made in subdued light, and a standard sample equivalent to a serum containing 16 mg. of PAS per 100 ml. was analyzed with each group of samples. The average concentration obtained with the known sample in some 143 determinations was 15.85 mg. per 100 ml., and the standard error was 0.06 mg.

The following drugs were studied as described above:

(*1*) PAS-ascorbate (Pascorbic tablets, 0.5 gm.—Hellwig)[8]
(*2*) Sodium PAS (plain compressed tablets, 0.5 gm.—manufacturer uncertain)
(*3*) Potassium PAS (capsules, 0.5 gm., Paskate®—Lilly #348)[8]
(*4*) Acid PAS buffered, 0.5 gm. (buffered Parasal® tablets, buffered with dihydroxy aluminum aminoacetate and calcium carbonate)
(*5*) Acid PAS enteric coated (Parasal tablets S-A, 1.0 gm.—Panray #743—0.5 gm. buffered and 0.5 gm. enteric coated)[8]
(*6*) Sodium PAS enteric coated (Teebacin® glycine buffered tablets—C.M.C.)[8]
(*7*) Acid PAS buffered, 0.5 gm. (Teebacin acid buffered, buffered with magnesium glycinate and magnesium carbonate)
(*8*) Acid PAS-resin combined (Rezipas® powder—Squibb)
(*9*) Acid PAS plain (Parasal tablets, 0.5 gm.—Panray #721)[8]

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[1] This investigation was supported in part by a grant from the Ohio Tuberculosis and Health Association.

[2] Donnerberg, R. L., *et al.*: Am. Rev. Tuberc., 1957, *76*, 877.

[3] Duncan, D. K., *et al.*: Dis. of Chest, 1951, *19*, 138.

[4] Riska, N.: Acta tuberc. Scandinav., 1954, *30*, Nos. 1–2.

[5] Hollander, A. G.: Am. Rev. Tuberc., 1955, *72*, 548.

[6] Deeb, E. N., and Vitagliano, G. R.: Am. Rev. Tuberc., 1955, *72*, 543.

[7] Pratt, P. C.: Am. Rev. Tuberc., 1957, *75*, 93.

[8] Drug contributed by manufacturer.

For drug *No. 1* (PAS-ascorbate) all 5 members of the group (*Group A*) completed the study. For *No. 2* only 3 of the group were available. Four of the 5 patients were studied with drugs *Nos. 3* and *4* (*see* tables 1–4 and figure 1).

Another group of 4 men (*Group B*) was selected. All were cooperative in completing 24-hour studies with drugs *Nos. 5, 6, 7, 8* and with *No. 2* (sodium PAS) repeated as a standard of reference (tables 5–9 and figure 2).

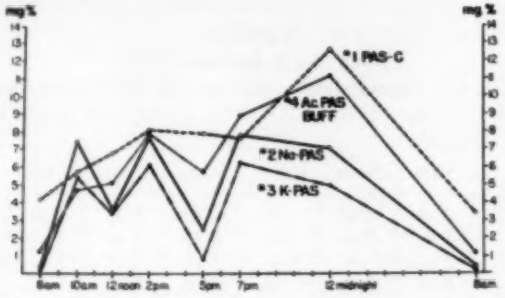

FIG. 1. Twenty-four hour studies of average serum concentrations of *Group A* patients.

TABLE 1

DRUG NO. 1: PAS-ASCORBATE

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	12 Mid-night	8 a.m.
WS	1.92	6.56	7.36	6.52	6.40	7.76	17.60	3.76
MC	2.16	7.32	6.28	13.12	8.40	7.32	12.32	2.76
RT	5.12	6.16	10.00	9.36	11.00	11.64	9.64	4.36
RL	10.08	7.48	5.08	4.48	7.72	6.32	11.56	5.96
CN	0.84	1.72	5.60	6.88	6.04	5.48	12.32	0.68
Average	4.02	5.85	6.86	8.07	7.91	7.70	12.69	3.50

TABLE 2

DRUG NO. 2: SODIUM PAS

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	12 Mid-night	8 a.m.
WS	0.48	7.92	3.80	9.16	2.40	9.64	8.76	0.44
MC	0.60	7.40	4.20	9.64	2.44	9.64	5.32	0.40
RT	—	—	—	—	—	—	—	—
RL	—	—	—	—	—	—	—	—
CN	0.24	7.88	3.08	3.88	2.52	4.00	6.92	0.36
Average	0.44	7.40	3.69	7.56	2.45	7.76	7.00	0.40

Still another group of 4 men (*Group C*) was treated under standard conditions with drugs *Nos. 9* (acid PAS) and *1* (PAS-ascorbate) repeated as a standard of reference, with 24-hour observation of serum levels (*see* tables 10 and 11 and figure 3).

In figure 1 are shown the average serum concentrations of *Group A* patients during a 24-hour study of drug *No. 1:* PAS-ascorbate; *No. 2:* sodium PAS; *No. 3:* potassium PAS; *No. 4:* acid PAS buffered. The data may be seen in tables 1, 2, 3, and 4. It is noted that

TABLE 3

DRUG NO. 3: POTASSIUM PAS

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	12 Midnight	8 a.m.
WS	0.48	5.00	3.72	7.60	1.52	5.64	6.56	0.24
MC	0.48	7.40	3.68	6.92	1.80	8.00	3.68	0.08
RT	—	—	—	—	—	—	—	—
RL	0.28	5.32	3.84	6.32	2.56	8.24	5.12	0.16
CN	0.12	3.88	2.84	3.84	1.52	3.20	4.60	0.24
Average	0.34	5.40	3.52	6.17	1.85	6.27	4.99	0.18

TABLE 4

DRUG NO. 4: BUFFERED PAS

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
WS	2.76	4.76	4.24	7.08	7.36	11.64	14.64	1.80
MC	0.84	6.64	5.16	7.56	5.28	6.88	10.60	0.92
RT	—	—	—	—	—	—	—	—
RL	0.84	4.76	5.40	7.72	5.84	8.32	11.40	1.12
CN	0.52	3.28	5.36	9.44	4.52	8.32	8.00	0.40
Average	1.24	4.86	5.04	7.95	5.75	8.79	11.16	1.06

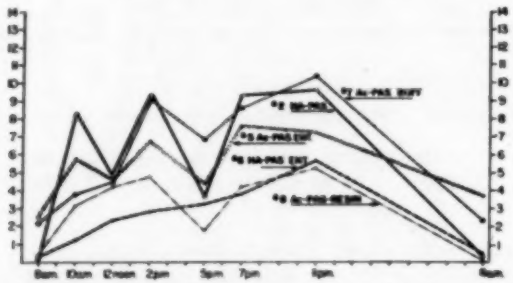

FIG. 2. Twenty-four hour studies of average serum concentrations of *Group B* patients.

serum concentrations for potassium PAS are the lowest in the group; that those for sodium PAS are somewhat higher at their peaks but were poorly sustained. The highest daytime concentrations were attained in patients taking PAS-ascorbate and acid PAS buffered. The highest single concentration was found with acid PAS-ascorbate administration: 12.69 mg. per 100 ml. at 12 midnight, 3 hours after the 9 p.m. dose. Only in the case of PAS-ascorbate was there a well-sustained overnight concentration: 3.50 mg. per 100 ml. at 8 a.m., 11 hours after the 9 p.m. dose.

TABLE 5

DRUG NO. 5: ACID PAS ENTERIC

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
CR	1.04	5.56	5.60	7.72	6.40	9.04	8.24	4.66
TJ	3.04	6.16	4.08	6.56	3.16	6.68	7.20	3.48
BS	2.40	6.22	2.80	6.22	4.24	6.72	5.60	1.08
RS	3.68	4.92	6.08	6.72	3.76	8.32	7.56	5.64
Average	2.54	5.72	4.64	6.81	4.39	7.69	7.15	3.72

TABLE 6

DRUG NO. 6: SODIUM PAS ENTERIC

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
CR	0.76	0.46	0.58	3.84	4.08	4.80	5.48	0.36
TJ	0.32	3.36	2.28	1.00	2.56	1.80	6.00	0.44
BS	0.12	0.24	4.16	3.48	3.00	1.32	8.08	0.16
RS	1.60	0.90	2.28	3.20	3.40	3.96	3.00	0.68
Average	0.70	1.24	2.33	2.88	3.26	2.97	5.64	0.41

TABLE 7

DRUG NO. 7: ACID PAS BUFFERED

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
CR	3.68	4.00	4.20	4.76	8.40	11.40	10.92	3.96
TJ	0.92	4.40	5.44	11.56	8.08	7.40	10.52	2.36
BS	0.24	2.84	3.76	11.48	5.92	8.40	8.92	0.16
RS	3.44	3.60	4.68	8.28	5.28	7.12	11.32	3.04
Average	2.07	3.71	4.52	9.02	6.92	8.58	10.42	2.38

Group B patients were studied in the same manner with drugs *No. 5:* acid PAS enteric coated; *No. 6:* sodium PAS enteric coated; *No. 7:* acid PAS buffered; *No. 8:* acid PAS-resin combined; and *No. 2:* sodium PAS plain. Average serum concentrations are demonstrated in figure 2, with supporting data in tables 5, 6, 7, 8, and 9.

The two lowest curves in figure 2 represent serum concentrations of patients who received PAS-resin and sodium PAS enteric coated. Even in the daytime, the concentrations barely reached 5 mg. per 100 ml., then dropped to nearly zero overnight. The curve for sodium PAS is similar to that of the same drug in figure 1, with rapid fluctuation and

TABLE 8

DRUG NO. 8: ACID PAS-RESIN

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
CR	0.28	1.92	2.64	4.56	2.76	3.28	6.36	0.20
TJ	—	—	—	—	—	—	—	—
BS	0.08	3.76	5.00	5.48	1.68	4.72	5.00	0.08
RS	1.52	3.76	5.48	4.24	0.96	4.48	3.72	0.12
Average	0.63	3.15	4.37	4.76	1.80	4.16	5.03	0.13

TABLE 9

DRUG NO. 2: SODIUM PAS

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
CR	0.68	4.96	5.00	8.76	4.68	5.36	11.72	0.72
TJ	0.72	10.28	4.32	8.52	4.24	13.36	11.08	0.52
BS	0.20	8.24	4.32	10.64	2.04	9.56	10.08	0.12
RS	0.28	9.44	5.36	9.08	3.24	9.04	6.04	0.20
Average	0.47	8.23	4.75	9.25	3.55	9.33	9.73	0.39

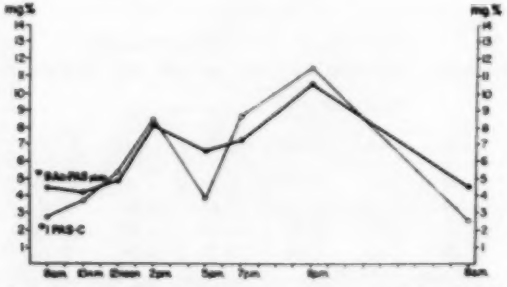

FIG. 3. Twenty-four hour studies of average serum concentrations of *Group C* patients.

TABLE 10

DRUG NO. 9: ACID PAS, PLAIN

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
RR	9.48	6.12	4.36	7.60	8.32	6.76	12.92	8.88
RL	0.24	0.96	4.56	5.24	5.60	6.08	6.56	0.28
BW	0.64	3.48	4.56	11.24	6.28	8.64	9.48	0.88
SY	7.40	6.08	6.40	8.04	6.08	7.00	12.96	7.68
Average	4.44	4.16	4.97	8.03	6.57	7.12	10.48	4.43

TABLE 11

DRUG NO. 1: PAS-ASCORBATE

PAS Serum Concentrations, Mg. per 100 Ml., 24-Hour Study

Patient	Time							
	8 a.m.	10 a.m.	12 Noon	2 p.m.	5 p.m.	7 p.m.	11 p.m.	8 a.m.
RR	7.04	4.12	2.68	4.92	3.48	7.12	10.28	6.24
RL	0.12	1.04	6.40	8.96	3.84	6.84	6.44	0.28
BW	0.72	3.32	4.40	12.36	7.24	9.32	12.88	1.00
SY	2.92	5.80	7.60	6.80	4.48	10.72	16.00	3.12
Average	2.70	3.57	5.27	8.26	4.76	8.50	11.40	2.66

poorly sustained night levels. Acid PAS buffered (*No. 7*) and acid PAS enteric coated (*No. 5*) each demonstrated a good daytime level with overnight concentrations which remained above 2 mg. per 100 ml.

Group C patients, also 4 men, received only two drugs for the 24-hour study: *No. 9:* acid PAS, plain tablets; *No. 1:* PAS-ascorbate tablets. Average serum levels for the group are seen in figure 3, based on tables 10 and 11. The curves are quite similar, each drug producing an average late evening peak above 10 mg. per 100 ml. and each one maintaining a satisfactory concentration throughout the night. Acid PAS concentrations at 8 a.m. were considerably higher than those with PAS-ascorbate: 4.43 mg. per 100 ml. as compared with 2.66 mg. per 100 ml. The PAS-ascorbate curve in *Group C* patients is similar to that in the *Group A* patients, except that the latter averaged a little higher. In *Group A*, one of 5 patients (*CN*) had low serum concentrations, whereas in *Group C* 2 of 4 patients (*RL* and *BW*) had low serum concentrations.

* * *

In reviewing these data, it is seen that the highest sustained drug concentrations during the night were attained with *No. 1:* PAS-ascorbate; *No. 5:* acid PAS buffered and enteric coated; *No. 7:* acid PAS buffered; and *No. 9:* acid PAS plain. All other forms of the drug demonstrated morning serum concentrations below 1.5 mg. per 100 ml., with most of them below 1.0 mg. per 100 ml. The sodium and potassium salts, the resin-combined form, and one of the buffered acid PAS products were all inferior with respect to

sustained serum concentrations. Several of this latter group demonstrated relatively low daytime concentrations. Sodium PAS, when given as plain tablets, revealed rapid absorption and disappeared from the blood stream more rapidly than some other forms.

The popularity of sodium PAS has been based on its relatively good tolerance by patients. This was shown in the first report of this series. Acid PAS and PAS-ascorbate as plain tablets are not as well tolerated at the 12 gm. daily dosage, but demonstrate higher average serum concentrations throughout the 24 hours of the day. Drugs *Nos. 5*, the buffered and enteric coated acid PAS, and *7*, the buffered acid PAS, both showed superior sustained serum concentrations overnight. Tolerance studies of these two products have not yet been carried out by the present writers, although obviously this information is needed.

In the day-to-day control of PAS administration, reduction in the dosage of PAS is common when the patient cannot tolerate the full daily dose (16.4 gm. sodium PAS = 12 gm. PAS). If the patient tolerates one-half the daily dose or more, the drug can be continued rather than replaced by another drug. Such a decision seems justified in the light of a recent United States Public Health Service cooperative study[9] comparing results in patients receiving full doses and half doses of sodium PAS. Naturally, one questions whether a reduced dose of PAS-ascorbate or one of the acid PAS products might be as well tolerated and produce as high serum concentrations as full doses of sodium PAS. Some evidence on this subject appeared in the first report. Further information regarding serum concentrations after administration of reduced doses will be presented in a third report.

It is apparent from the results demonstrated that there are tremendous variations in serum concentrations of PAS in different subjects and with different forms of the drug in the same patients. Some of these compounds produce high daytime concentrations but leave the blood stream more rapidly than others, so that during part of the night serum concentrations are below the ordinarily accepted therapeutic standard of 1.5 to 2.0 mg. per 100 ml. It should be remembered that the last dose of the day in these studies was at 9 p.m. In many tuberculosis hospitals (perhaps in most), the last dose is at supper time, about 5 p.m., thus allowing a longer period during which nontherapeutic serum levels may exist.

To the present writers' knowledge, nothing has been reported regarding the therapeutic importance of consistent serum concentrations above 2 mg. per 100 ml. as compared with concentrations which decline to presumably insignificant levels during the night. Neither is it known whether that most important function of PAS, namely, the delaying of resistance among companion drugs, is influenced adversely by low nocturnal PAS serum concentrations.

Observations during this study indicate that serum concentrations reach their maximum within 24 to 48 hours after PAS administration is started. Whether tissue concentrations follow closely or lag many hours or days behind, is unknown. Further studies soon to be undertaken may shed some light on this question.

In summary: Comparison of blood serum concentrations indicates a great variation in absorption rate of different forms of PAS. Likewise, the rate of disappearance of the drug from the blood stream, whether by metabolism or excretion, varies with the form of PAS.

[9] United States Public Health Service Cooperative Clinical Investigation of Antimicrobial Therapy of Tuberculosis: Study #7, Therapeutic Efficacy of Pyrazinamide.

Generally speaking, the best sustained and most consistent serum concentrations were found with PAS-ascorbate and acid PAS. The commonly used sodium salt of PAS is absorbed promptly, but serum concentration drops rapidly so that there are wide fluctuations, with a level approaching zero in the early morning.

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THE DEVELOPMENT OF LEUKOCYTIC SENSITIVITY TO TUBERCULIN IN GUINEA PIGS EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM TUBERCULOSIS H37Rv[1]

A number of excellent studies have been made in which the leukocytes of tuberculous animals have been found to be sensitive to tuberculin *in vitro*. In these studies a very high degree of correlation between skin and leukocytic sensitivity has been consistently observed. *In vitro* studies using human leukocytes,[2,3] on the other hand, have revealed that there is a considerably lower degree of correlation between the two sensitivities than that found in animals. Furthermore, the correlation has been found to be poorest among those subjects with minimal tuberculosis[2].

The present study was undertaken to test the possibility that the observed lack of correlation between skin and leukocytic sensitivity in humans might be due to differences in the abilities of the two methods (intradermal injection of tuberculin and migration inhibition) to detect sensitivity or to differences in the time of appearance of skin and leukocytic sensitivity.

Two groups of 20 tuberculin skin-test-negative guinea pigs were chosen at random from the departmental stock. One group was injected intramuscularly with 0.01 mg. wet weight of *Mycobacterium tuberculosis* H37Rv cells; the other group was used as a normal control. The sensitivities of the skin and the leukocytes of both groups were tested at intervals from the time of injection until the leukocytes of the animals in the infected group had developed a marked degree of specific tuberculin sensitivity.

Old Tuberculin (OT) in dilutions of 1:100, 1:1,000, and 1:10,000 was used to test for skin and leukocytic sensitivity. Skin sensitivity was determined by intradermal injection, and leukocytic sensitivity was determined by a modification of the tissue culture method previously described.[2] The degree of leukocytic sensitivity was calculated using the method of Heilman, Feldman, and Mann.[4]

Results with normal controls: Twenty-four tests were made with the leukocytes of 16 different guinea pigs using each of the three dilutions of OT. An analysis of the comparative cytotoxic indices (CCI) obtained is given in table 1. The range of indices was from 0.69 to 1.30 with a mean ($\bar{X}$) of 0.9991 and a standard deviation of 0.1066 for the 1:100 dilution; 0.84 to 1.16 with a mean of 1.0008 and a standard deviation of 0.075 for the 1:1,000 dilution; and 0.88 to 1.12 with a mean of 0.9988 and a standard deviation of 0.0477 for the 1:10,000 dilution. Individual indices obtained with the leukocytes of infected animals were considered to be indicative of specific tuberculin sensitivity if they fell in the ranges below $\bar{X} - 2S$ (0.79 for 1:100; 0.85 for 1:1,000; 0.90 for 1:10,000).

Results with infected guinea pigs: The tuberculin skin test reactions and the comparative cytotoxic indices obtained with the leukocytes of the infected guinea pigs are given in tables 2, 3, and 4.

With the 1:100 dilution of OT the mean comparative cytotoxic indices dropped sharply the first week to 0.83, and this downward trend was maintained at a somewhat more gradual rate until a low of 0.66 was reached in the sixth week. All of the skin test reactions were negative the first week; one was negative, three were weakly positive, and

[1] This study was supported by a research grant, No. H-2422, from The National Institutes of Health, U. S. Public Health Service.

[2] O'Neill, E. F., and Favour, C. B.: Am. Rev. Tuberc., 1955, *72*, 577.

[3] Hall, H. E., and Scherago, M.: Am. Rev. Tuberc., 1957, *75*, 807.

[4] Heilman, D. H., *et al.*: Am. Rev. Tuberc., 1944, *50*, 344.

TABLE 1

ANALYSIS OF THE COMPARATIVE CYTOTOXIC INDICES OF THE NORMAL GUINEA PIGS

Dilution of OT	Number of Animals	Number of Tests	Range of CCI	$\bar{X}$	S	$\bar{X} - 2S$
1:100	16	24	0.69–1.30	0.9991	0.1066	0.7859
1:1,000	16	24	0.84–1.16	1.0008	0.0740	0.8528
1:10,000	16	24	0.88–1.12	0.9988	0.0477	0.9034

CCI—Comparative cytotoxic indices.
$\bar{X}$—Mean
S—Standard deviation.

TABLE 2

SKIN TEST RESULTS AND COMPARATIVE CYTOTOXIC INDICES (CCI) OF THE TUBERCULOUS GUINEA PIGS, USING OLD TUBERCULIN, 1:100

Week After Infection																				
First			Second			Third			Fourth			Fifth			Sixth			Eighth		
A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L
1	−	0.79	11	−	0.81	4	+	0.77	2	+	0.70	1	+	0.76	4	+	0.87	2	+	0.79
2	−	0.77	12	±	0.70	9	−	0.87	6	+	0.68	12	+	0.61	15	+	0.61	7	+	0.50
6	−	0.87	23	±	0.92	15	+	0.74	7	+	0.76	23	+	0.62	23	+	0.62	35	+	0.72
7	−	0.92	28	±	0.77	22	+	0.69	21	±	0.58	31	+	0.77	30	+	0.69	39	+	0.73
35	−	0.81	31	+	0.70	30	±	0.78	35	+	0.84	39	+	0.72	33	+	0.70			
			39	+	0.84	32	−	0.75												
Mean 0.82			Mean 0.80			Mean 0.77			Mean 0.71			Mean 0.70			Mean 0.66			Mean 0.68		

Note: Individual CCI below 0.79 are indicative of specific tuberculin sensitivity.
A—Animal number. Sk—Skin sensitivity. L—Leukocyte sensitivity (CCI). − Negative. ± Weakly positive. + Strongly positive.

two strongly positive the second week; two were negative and four strongly positive the third week; and all were strongly positive the subsequent weeks.

Two animals with negative tuberculin skin reactions had indices in the range of specific tuberculin sensitivity, *No. 2* (CCI 0.77) the first week and *No. 32* (CCI 0.75) the third week. On the other hand, 3 animals with positive tuberculin skin reactions failed to show indices in the range of specific tuberculin sensitivity: *No. 23* (CCI 0.92), *No. 39* (CCI 0.84) the second week, and *No. 35* (CCI 0.84) the fourth week. By the fifth week after infection all of the animals tested had indices in the range of specific tuberculin sensitivity.

With the 1:1,000 dilution of OT the mean comparative cytotoxic indices remained constant (1.03) for the first two weeks after infection and then dropped rapidly, reaching a low of 0.61 in the fifth week. All of the skin test reactions were negative the first week; four were negative and two weakly positive the second week; four were negative, one weakly positive, and one strongly positive the third week; three were weakly positive and two strongly positive the fourth week; and all were strongly positive the subsequent weeks. Three animals with negative tuberculin skin reactions had indices in the range of specific tuberculin sensitivity: *No. 11* (CCI 0.78) and *No. 28* (CCI 0.83) the second week, and *No. 22* (CCI 0.81) the third week. Three animals with weakly positive tuberculin skin reactions had high indices: *No. 31* (CCI 1.11) and *No. 39* (CCI 1.26) the second week,

TABLE 3

SKIN TEST RESULTS AND COMPARATIVE CYTOTOXIC INDICES (CCI) OF THE TUBERCULOUS GUINEA PIGS, USING OLD TUBERCULIN, 1:1,000

Week After Infection																				
First			Second			Third			Fourth			Fifth			Sixth			Eighth		
A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L
1	−	1.10	11	−	0.78	4	+	0.77	2	+	0.75	1	+	0.60	4	+	0.55	2	+	0.78
2	−	1.00	12	−	1.10	9	−	0.96	6	±	0.79	12	+	0.56	15	+	0.53	7	+	0.43
6	−	1.05	23	−	1.07	15	±	0.80	7	±	1.00	23	+	0.52	22	+	0.73	35	+	0.66
7	−	1.11	28	−	0.83	22	−	0.81	21	±	0.67	31	+	0.70	30	+	0.75	39	+	0.82
35	−	0.87	31	±	1.11	30	−	1.06	35	+	0.83	39	+	0.67	32	+	0.73			
			39	±	1.26	32	−	0.97												
Mean 1.03			Mean 1.03			Mean 0.90			Mean 0.83			Mean 0.61			Mean 0.66			Mean 0.67		

Note: Individual CCI below 0.85 are indicative of specific tuberculin sensitivity.

A—Animal number. Sk—Skin sensitivity. L—Leukocyte sensitivity (CCI). − Negative. ± Weakly positive. + Positive.

TABLE 4

SKIN TEST RESULTS AND COMPARATIVE CYTOTOXIC INDICES (CCI) OF THE TUBERCULOUS GUINEA PIGS, USING OLD TUBERCULIN, 1:10,000

Week After Infection																				
First			Second			Third			Fourth			Fifth			Sixth			Eighth		
A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L	A	Sk	L
1	−	0.99	11	−	1.03	4	−	0.72	2	+	0.67	1	±	0.84	4	+	0.75	2	+	0.73
2	−	0.88	12	−	0.93	9	−	1.06	6	−	1.02	12	±	0.68	15	+	0.83	7	+	0.67
6	−	0.98	23	−	0.85	15	−	0.90	7	−	1.05	23	±	0.90	22	+	0.77	35	+	0.73
7	−	1.09	28	−	0.90	22	−	0.83	21	±	0.78	31	+	0.84	30	+	0.90	39	+	0.82
35	−	1.08	31	−	1.16	30	−	0.88	35	±	0.89	39	+	0.92	32	+	0.85			
			39	−	1.08	32	−	0.92												
Mean 1.00			Mean 0.90			Mean 0.93			Mean 0.88			Mean 0.84			Mean 0.78			Mean 0.74		

Note: Individual CCI below 0.90 are indicative of specific tuberculin sensitivity.

A—Animal number. Sk—Skin sensitivity. L—Leukocyte sensitivity (CCI). − Negative. ± Weakly positive + Positive.

and *No. 7* (CCI 1.09) the fourth week. By the fifth week after infection all of the animals tested had indices in the range of specific tuberculin sensitivity.

With the 1:10,000 dilution of OT the mean comparative cytotoxic indices remained fairly constant for the first two weeks after infection and then dropped gradually to a low of 0.74 by the eighth week. All of the skin test reactions were negative for the first three weeks; two were negative, two weakly positive, and one strongly positive the fourth week; three were weakly positive and two strongly positive the fifth week; and all were strongly positive the subsequent weeks. Seven animals with negative tuberculin skin reactions had indices in the range of specific tuberculin sensitivity: *No. 2* (CCI 0.88) the first week, *No. 23* (CCI 0.85) and *No. 28* (CCI 0.90) the second week, *No. 4* (CCI 0.72), *No. 15* (CCI 0.90), *No. 22* (CCI 0.83), and *No. 30* (CCI 0.88) the third week. Only 2 animals with positive tuberculin skin reactions failed to show specific tuberculin sensitivity: *No. 39* (CCI 0.92) the fifth week and *No. 30* (CCI 0.98) the sixth week. Not until

the eighth week were all of the indices of the animals tested in the range of specific tuberculin sensitivity.

* * *

These results indicate that the leukocyte migration inhibition technique was more capable of detecting tuberculin sensitivity in the experimentally infected guinea pigs than the tuberculin skin test. Only 8 (12 per cent) of the 68 migration inhibition tests on the leukocytes of the guinea pigs with positive tuberculin skin reactions failed to show leukocytic sensitivity, while 12 (30 per cent) of the 40 migration inhibition tests on the guinea pigs with negative tuberculin skin reactions had indices indicative of leukocytic sensitivity. Leukocytic sensitivity appeared earlier in most instances than did skin sensitivity, as indicated by the low mean comparative cytotoxic indices obtained with the 1:100 dilution of OT the first week and with the 1:10,000 dilution the third week. In both cases the skin tests were all negative. The mean indices, however, had dropped sharply from the normal level to 0.83 with the 1:100 dilution and to 0.88 with the 1:10,000 dilution. However, the results fail to account for the lack of correlation between the sensitivities of the skin and the leukocytes of humans to tuberculin[2,3].

In summary: The skin and leukocytic sensitivities of a group of guinea pigs experimentally infected with *M. tuberculosis* strain H37RV have been compared with those of an uninfected control group from the time of infection of the former until their leukocytes showed marked specific tuberculin sensitivity.

The migration inhibition technique was found to be more capable of detecting tuberculin sensitivity than the intradermal skin test. In most instances the leukocytic sensitivity developed earlier than the skin sensitivity.

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PRELIMINARY OBSERVATIONS WITH THE USE OF PAPER ELECTROPHORESIS IN STUDYING SERUM PROTEIN CHANGES AS AN INDEX OF PROGRESS IN THE TUBERCULOUS PATIENT

Alteration of the normal serum protein pattern in the tuberculous patient, proportionate to the extent and activity of the disease, has been observed by numerous investigators.[1] Serial protein fraction studies conducted during treatment for the purpose of supplementing and confirming other clinical data have received far less attention, yet the value of such analyses been demonstrated.[2]

Recognized limitations of the classic Tiselius technique apparently have restricted its extensive application for other than research or strictly diagnostic purposes. Recent advances in paper electrophoresis, especially with reference to improvements in quantitation,[3] prompted consideration of the procedure as a practical approach to determination of the individual protein fractions. The potential value of such data can be determined only by the extent of its contribution to the final evaluation of a patient's progress. Thus, correlation with a clinical study is required, and the cycloserine pilot study in progress was selected for this investigation.

Eight patients with moderate to far advanced active pulmonary tuberculosis were observed while on cycloserine therapy, 1 gm. daily, for a period of seven months. Patients receiving cycloserine were chosen as it was believed that this drug had not been used previously in any of these 8 cases. Four patients had received no previous chemotherapy, and 4 were receiving chemotherapy again after having had antimicrobials over a long interval of time without any clinical improvement and with the appearance of drug-resistant infections. All of these patients had studies of renal and hepatic function and examination of the sputum for tubercle bacilli at fortnightly intervals.

The protein fraction determinations were done at intervals throughout the study by the procedure of Durrum.[3] Ten microliter (0.01 ml.) samples of the serum were applied to the apex of 3 MM filter paper strips in the Durrum migration chamber and six milliamperes of current passed through the eight strips for seventeen hours, using barbiturate buffer pH 8.6, ionic strength 0.075. After the coagulation of the migrated fractions (thirty minutes at 125°C.), the strips were dyed overnight in 0.01 per cent bromphenol blue, rinsed, fixed, and dried. In this series the strips were allowed to achieve a stable color by equilibration with moisture in the air. (This step has recently been improved through increase of the sodium acetate concentration of the fixing bath and exposure to ammonia vapor just prior to scanning.) The scanning and automatic integration of the strips were conducted with the Specialized Instruments Corporation Analytrol.

It is emphasized that a standardized technique was rigidly maintained to achieve maximal reproducibility. It should be pointed out that to date there has been one variable factor, which is lack of uniformity in the filter paper medium. It is possible that in the near future an improved type of filter paper may be made available which may increase the accuracy and reproducibility of the method.

The total protein values of the serum were within normal to slightly above normal range. The ratio of albumin to total globulin, calculated from the electrophoresis values,

[1] GUTMAN, A. B.: In *The Plasma Proteins in Disease, Advances In Protein Chemistry*, Vol. 4, Academic Press, Inc., New York, 1948, p. 212.

[2] VOLK, B. W., *et al.*: Am. Rev. Tuberc., 1953, *67*, 299.

[3] BLOCK, R. J., *et al.*: In *A Manual of Paper Chromatography and Paper Electrophoresis*, Academic Press, Inc., New York, 1955, Chap. 18.

was used as a factor in reflecting the changes in serum proteins as they occurred during treatment.

In the first group of 4 patients (previously untreated), clinical improvement became manifest within ten weeks by reversal of infectiousness, a definite decrease in symptoms, cough, expectoration, and an increase in weight. Serial roentgenograms of the chest revealed noticeable improvement. The pretreatment protein fractions in this group exhibited elevated alpha and gamma globulins associated with a reduction in albumin. The initial electrophoretic ratio determined from the strips ranged from 0.57 to 1.17 for the 4 patients.

In three cases the protein values began to show improved patterns soon after the start of treatment, with a close approach to normal values after four to six months of treatment. The protein pattern in the fourth case started to improve after twelve weeks of treatment. Changes in the electrophoretic albumin-globulin ratio during therapy are presented in the graph for the 4 previously untreated patients. Three patients (*B. J.*, *S. G.*, and *M. S.*), who showed an improved electrophoretic albumin-globulin pattern and who maintained such a pattern, had an excellent clinical course with roentgenographic improvement and reversal of infectiousness. These patients were subsequently discharged from the hospital with the diagnosis of inactive disease. The fourth (*A. C.*), who showed an excellent response only to have a decline in his electrophoretic albumin-globulin pattern, proceeded on to excellent clinical recovery, with roentgenographic improvement and reversal of infectiousness. In this case however, it is believed that his electrophoretic albumin-globulin pattern decline was probably due to a state of uncontrollable diabetes, which he had at that time.

In the group of previously treated patients, transient improvement in the symptoms, with slight weight gain, was noted early. However, sputum examinations, including cultures, remained positive for tubercle bacilli in 3 cases. In the fourth case, the sputum became negative for tubercle bacilli after the third month of therapy. Serial roentgenograms of the chest revealed no significant change in 2 patients, one showed moderate

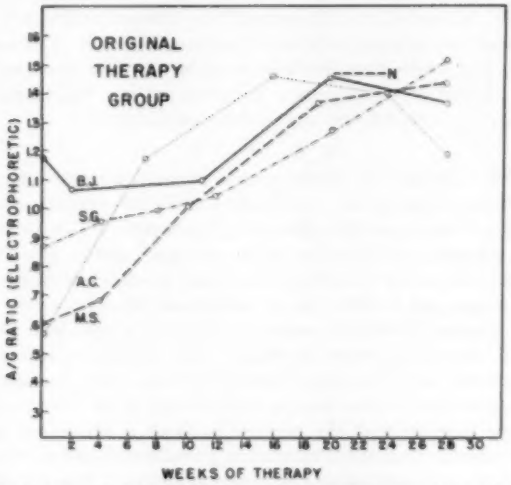

FIG. 1. Graph showing clinical improvement of 4 intially treated patients.

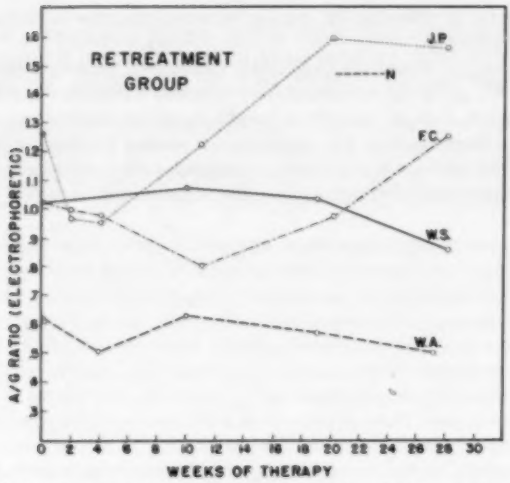

FIG. 2. Graph showing transient improvement in 4 patients previously treated with antimicrobial drugs.

progression of the disease, and, in the fourth patient whose sputum became negative for tubercle bacilli, roentgenographic improvement occurred.

The initial serum protein fractions at the start of treatment exhibited elevated alpha globulins, probably indicating the extent of tissue involvement, as well as increased gamma globulin. The electrophoretic albumin-globulin ratios ranged from 0.62 to 1.25.

After six months of treatment, the protein fractions remained at about the same levels, with no change in the electrophoretic albumin-globulin ratios seen previously in patients *F. G.*, *W. S.*, and *W. A.* The course of the disease in patient *W. S.* was entirely unfavorable and his roentgenograms remained unchanged; but *W. A.* showed slight clinical improvement for four weeks, then deterioration of his condition was manifest. The fourth patient (*J. P.*) showed roentgenographic improvement and noninfectious sputum, and exhibited improved protein patterns approaching normal levels.

* * *

An attempt has been made to demonstrate a correlation between serum protein fractions and the course of pulmonary tuberculosis while the patients were on cycloserine therapy. The serial electrophoretic albumin-globulin ratio, computed from the electrophoresis serum patterns, was found to be an accurate index in following the course of pulmonary tuberculosis under treatment. It was also possible to evaluate the efficacy of a new therapeutic agent and its effect in two comparable groups of cases. In one group the gradual return of serum proteins to normal levels would suggest that the new agent had some therapeutic value in previously untreated cases. The lack of response to the drug in the previously treated cases was demonstrated both clinically and also by failure of serum protein fractions to return to normal levels except in the one case described above.

Electrophoretic separation of the protein fractions is recognized as a standard of accuracy in the determination of the albumin to globulin ratio *and permits a reproducibility* not always available with existing routine chemical methods. This reproducibility has not been appreciably sacrificed through use of the technically simpler and less expensive paper

migration procedure for achieving the separation. The electrophoretic albumin-globulin ratio provides a summation in one factor of the recognized alterations in the serum protein fractions.

Consideration of the individual fractions contributes additional valuable information since alteration in the alpha globulins is the first to occur and may precede other clinical findings. This is followed by delayed alterations in the gamma fraction. Beta globulin has been reported to be elevated in pulmonary tuberculosis. This was not consistently seen in the present studies, and it is believed that a relative increase in this fraction is suggestive of hepatic dysfunction.

While this study is preliminary in nature, indications are encouraging that analysis of the protein fractions on paper strips may provide additional desirable information in the evaluation of chemotherapeutic agents and the clinical course of tuberculosis, as well as help prognosticate the end results of such treatment.

In summary: Paper electrophoresis provides accurate resolution of protein fractions which can be used to qualitatively appraise the progress of a tuberculous infection in a patient as well as determine an accurate index of inflammatory activity. These estimations may even precede other clinical measures of progress or regression. Although this is a preliminary report, further work in this field is encouraging in that the protein fractions by migration on paper are a valuable qualitative index of a patient's progress and will be reported later.

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March 15, 1957

FAILURE OF A METHOD FOR ENZYMATIC DIGESTION AND CONCENTRATION OF PATHOGENIC FUNGI AND MYCOBACTERIUM FROM SPUTUM

Several methods are now available for the concentration of tubercle bacilli in clinical specimens preparatory to culturing this organism. However, no specific method has been developed for the concentration of pathogenic systemic fungi.

This study was undertaken to investigate the possibility of an enzymatic digestion of specimens which would not only concentrate the small number of fungi present, but at the same time would maintain their viability and that of the Mycobacterium while destroying or inhibiting other bacteria.

The organisms tested included *N. asteroides*, *C. albicans*, *C. neoformans*, *M. tuberculosis*, and the yeast phase of *H. capsulatum* and *B. dermatitidis*. Each organism was "seeded," in a concentration of 5×10^2 cells per ml., into pooled normal sputum which contained predominately streptococci, Neisseria, and diphtheroids. These specimens were concentrated by the addition of equal portions of a 1 per cent trypsin solution (pH 6.0), homogenated by manual shaking, and placed into a 37° C. incubator. Liquefaction was usually complete within one hour. The specimen was then centrifuged at 2,000 r.p.m. for ten minutes and the sediment was inoculated onto Löwenstein-Jensen, Thompson cysteine blood agar, and Sabouraud glucose agar media. Trypsin was found to have no deleterious effect on any test organism or bacterial flora present in the specimen.

In order to inhibit contaminating bacterial flora, the trypsin digestant was prepared in dilutions of Zephiran® chloride, G-4,[1] and Ceepryn® chloride. These dilutions included 1:1,000, 1:2,000, 1:4,000, 1:8,000, and 1:10,000 of each germicidal solution. It was found that a dilution of 1:1,000 of each of the solutions inhibited the growth of all organisms except *M. tuberculosis* after one hour of incubation. A solution containing 1 per cent trypsin and 1:4,000 Zephiran chloride was inhibitory to *B. dermatitidis* and *H. capsulatum*, but had no appreciable effect upon other test organisms or bacterial flora. No one dilution of Zephiran, G-4, or Ceepryn chloride used was found to inhibit bacterial flora without also affecting the test organisms. Digestion of the Löwenstein-Jensen medium took place in the trypsin controls and in experiments in which the germicidal agents were diluted more than 1:4,000.

To enhance the mucolytic properties of trypsin, the digestant was prepared in a 0.7 per cent sodium hydroxide solution (pH 8.5). This digestant, including the 1:10,000 dilution of Zephiran, inhibited the growth of all organisms tested except *M. tuberculosis* and *N. asteroides*. Neutralization of the alkaline digestant mixture after thirty minutes' incubation was without effect on the destructive action of the mixture.

From the observations made in this study, it was found that trypsin alone had no action upon the intact living fungus cell. Chemical inhibitors of bacterial growth in general were unfortunately equally effective against the fungi at levels in which bacterial growth was inhibited. Perhaps if some agent can be found which will not affect the pathogenic systemic fungi, but at the same time will inhibit the growth of contaminating bacteria, a suitable method will evolve for the concentration of fungi from infected materials.

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April 8, 1957

[1] 2,2[1] methylenebis-(4 chlorophenol), manufactured by Sindar Co.

PATHOGENESIS OF MEDIASTINAL EMPHYSEMA COMPLICATING THERAPEUTIC PNEUMOPERITONEUM

Mediastinal emphysema complicates therapeutic pneumoperitoneum on rare occasions. The mechanism by which this complication arises has been assumed without actual proof to depend upon postulated defects or rupture of tissues between the peritoneal cavity and the mediastinum. However a simpler pathogenesis has been reported by the writer in previous publications[1, 2] and confirmed by Peabody and Buechner.[3] This consists of the dissection of air, accidentally deposited in the anterior abdominal wall, along

FIG. 1. Postero-anterior chest film showing aberrant air along the left cardiac border.

the fascial planes which are continuous between the abdominal wall and the anterior mediastinum. This can be demonstrated by means of a lateral roentgenogram which includes the abdomen as well as the chest.

The route of this dissection was thus revealed in two of three previously reported cases and has again been shown in a recent case briefly described below.

The patient was a 35-year-old female with moderately advanced active pulmonary tuberculosis. Pneumoperitoneum was initiated with 500 cc. of air injected in the usual manner with the needle placed at the level of the umbilicus at the left rectal margin. The initial pressure was not obtained by the operator but the pressure rose to a maximum of 10 cm. of water with each 100 cc. of air administered. The patient complained of some pain

[1] WEISS, W., AND STANLEY, C. O.: J. Phila. Gen. Hosp., 1951, *2*, 13.
[2] KOHOUT, F. W., *et al.*: Arq. clín., 1951, *13*, 55.
[3] PEABODY, J. W., JR., AND BUECHNER, H. A.: Am. Rev. Tuberc., 1953, *68*, 775.

FIG. 2. Lateral film revealing aberrant air in the anterior abdominal wall and anterior mediastinum.

at the site of injection, the epigastrium, and both shoulders, on arising from the table. She was permitted to walk around and, when seen the following morning, complained of pain in the midsternal region as well. At this time crepitus was noted in the anterior abdominal wall but none was found in the neck. A precordial systolic crunch was heard and roentgenograms were ordered.

The postero-anterior chest film showed aberrant air along the left cardiac border (figure 1), and the lateral film (figure 2) revealed this to be located anterior to the heart. The air could also be seen in the deeper layers of the anterior abdominal wall, and there was only a small amount of air in the peritoneal cavity under the diaphragms. The patient's symptoms were allowed to subside over a period of several days and pneumoperitoneum was resumed without incident.

In summary: In the writer's experience with 4 cases of mediastinal emphysema following artificial pneumoperitoneum, lateral chest roentgenograms in 3 cases have revealed the dissection of air from the anterior abdominal wall along the fascial planes into the anterior mediastinum. It is believed, therefore, that this is the usual mechanism by which the complication occurs. An excellent discussion of this subject has been presented recently by Peabody and Buechner.[3]

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April 9, 1957

THE EFFICIENCY OF CENTRIFUGATION AS A MEANS OF CONCENTRATING TUBERCLE BACILLI FROM SPUTUM[1]

It has been shown repeatedly that the probability of demonstrating tubercle bacilli in sputum of tuberculous patients is greatly increased when care is taken to select caseous particles for examination.[2] When for various reasons this is impractical, some method of "concentration" of the bacilli is often practiced. This usually involves digestion of the sputum with acid or alkali, followed by centrifugation and examination of the sediment. A number of investigators have reported on the effects of various methods of digestion and homogenization. Among others, Klein and associates[3] have studied the effect of centrifugation. They used a standard swinging cup centrifuge operated at speeds of 2,000 and 3,000 r.p.m. and planted aliquots of sediment and supernate on slants of Löwenstein medium. They found some gain in the total number of specimens from which bacilli were recovered and an approximate twofold increase in the number of colonies appearing in the positive tubes as the result of centrifugation.

This study was undertaken in an effort to define more precisely the extent to which tubercle bacilli are actually concentrated in the sediment when standard clinical procedures are used, and to determine to what extent more satisfactory results could be achieved by the use of high speed angle centrifugation.

Sputum, in which bacilli could be demonstrated by direct smear, was obtained from patients at the Brecksville Veterans Administration Hospital. It was pooled, divided into aliquots of approximately 50 ml., and mixed with an equal volume of 15 per cent solution of Na_3PO_4.[4] After standing at room temperature overnight the mixture was neutralized with 2N HCl, and 20 ml. aliquots were centrifuged. After centrifugation a portion of the supernate was aspirated, with care not to disturb the sediment, and was saved. The remainder of the supernate was then aspirated as completely as possible and discarded, and the sediment was resuspended in the original volume of fluid. Serial dilutions of sediment, supernate, and uncentrifuged material were prepared in TB broth (Difco) and planted on TB agar (Difco) plates. Colonies were counted after six weeks' incubation at 37° C.

Three centrifuges were used: (*1*) "International" Size 1 centrifuge with swinging tube carriers, operated for fifteen minutes at 3,000 r.p.m. (rcf. of approximately 1,800 g), (*2*) "International" Size 1 with angle head operated for fifteen minutes at 4,300 r.p.m. (2,700 g), (*3*) "CSI" centrifuge with angle head operated one hour at 11,000 r.p.m (14,000 g).

The number of bacilli per milliliter of digested sputum varied between 1.5×10^4 and 1.6×10^6. For ease of analysis the results of centrifugation are presented as percentage recovery of bacilli in the resuspended sediment, referred in each case to the bacilli per milliliter of original material as 100. It will be seen from comparison of the data presented in tables 1, 2, and 3 that within the limits of error of the method of counting them, practically all of the bacilli in the original homogenate could be accounted for in the resuspended sediment. Paradoxically the apparent recovery of bacilli from the sediment thrown down during high speed centrifugation was much less. Controlled experiments indicated that the apparent loss of bacilli was not due to heating of the centrifuge head

[1] Work carried out in partial fulfillment of the Project Teaching Program of the Medical Curriculum, Western Reserve University, School of Medicine, Cleveland, Ohio.

[2] Splendlove, G. A., *et al.*: Pub. Health Rep., 1949, *64*, 574.

[3] Klein, G. C., *et al.*: Am. J. Clin. Path., 1952, *22*, 581.

[4] Corper, H. J., and Stoner, R. E.: J. Lab. & Clin. Med., 1946, *31*, 1364.

TABLE 1

PERCENTAGE RECOVERY OF BACILLI IN RESUSPENDED SEDIMENT, FOLLOWING CENTRIFUGATION FOR 15 MINUTES AT 1,800 g

Trial	Per Cent Recovery of Resuspended Sediment
A	106.0
B	63.7
C	122.0
D	71.0
E	128.0
F	112.0
Average	100.5
Standard deviation	24.4

TABLE 2

PERCENTAGE RECOVERY OF BACILLI IN RESUSPENDED SEDIMENT, FOLLOWING CENTRIFUGATION FOR 15 MINUTES AT 2,700 g

Trial	Per Cent Recovery of Resuspended Sediment
G	67.5
H	80.0
I	95.0
J	125.0
K	109.0
L	36.3
M	109.0
Average	92.9
Standard deviation	22.9

TABLE 3

PERCENTAGE RECOVERY OF BACILLI IN RESUSPENDED SEDIMENT, FOLLOWING CENTRIFUGATION FOR 60 MINUTES AT 14,000 g

Trial	Per Cent Recovery of Resuspended Sediment
O	29.00
P	54.00
Q	68.50
R	20.00
S	51.00
T	95.00
Average	52.90
Standard deviation	24.75

during the process of centrifugation. It was concluded, therefore, that the apparent loss was due to packing of the bacilli into clumps from which they were not dispersed during the plating procedure.

* * *

The results of the study indicate that the efficiency of recovery of bacilli from sputum digested with trisodium phosphate is of a high order. Using standard clinical equipment

and procedures, essentially all of the viable bacilli present in the original homogenate could be found in the sediment. This conclusion is apparently at variance with that of other workers who found that little was to be gained by centrifuging homogenized sputum. The explanation probably lies in the nature of the methods used and the materials subjected to examination. In the studies reported here, all of the sputum specimens were rich in bacilli, and yielded positive cultures even after considerable dilution. In the previous work individual sputum specimens were examined, some of which were as rich in bacilli as the ones used in this study, and some were negative. Comparison was made of the number of cultures positive after direct planting of the homogenized sputum and the number positive when the sediment following centrifugation was planted. Only those specimens containing a "borderline" number of bacilli could be expected to be negative on direct planting and positive after centrifugation, or to give different colony counts under these conditions. Taking the results of both studies into consideration, it appears reasonable to conclude that centrifugation does achieve a high degree of concentration of bacilli, but that only in an occasional instance will this concentration give a positive result when a negative result might have been expected had uncentrifuged material been planted. The lack of increase in the number of "positives," therefore, is due not so much to an inadequate method as to the nature of the material being examined.

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April 24, 1957

PROBLEMS IN THE SURGICAL MANAGEMENT OF PULMONARY TUBERCULOSIS[1]

As long as surgery has been used in the treatment of pulmonary tuberculosis, there have been differences of opinion in regard to the indications for surgery as well as to many aspects of surgical technique.

A survey of some current differences was carried out, making use of the reporting mechanism established by the United States Veterans Administration and Armed Forces for studies on the chemotherapy of tuberculosis. Each hospital engaged in these studies was invited to report on the following surgical problems in the treatment of pulmonary tuberculosis: (*1*) the use of space-filling procedures with pulmonary resection; (*2*) the use of added drug coverage for resection in patients with cavities and/or bacillary resistance; (*3*) the indications for the resection of "closed" lesions; (*4*) the indications for the use of definitive collapse therapy (thoracoplasty and extraperiosteal plombage).

The comments of twenty-eight hospitals on these problems, in whole or in part, appeared in the October issue of *The Quarterly Progress Report of the Veterans Administration-Armed Forces Study on the Chemotherapy of Tuberculosis* (Volume XI, No. 3), and comments were received subsequently from sixteen additional hospitals. Hospitals reporting included the Fitzsimons Army Hospital, the St. Albans Naval Hospital, the Parks Air Force Base Hospital, and forty-one V. A. hospitals, a total of forty-four.[2]

A tabulation of replies on the various questions follows. Since all hospitals did not answer every question, discrepancies appear in all totals. A few explanatory comments are included but no attempt has been made to draw conclusions.

Preferences as to Methods for Reducing the Size of the Hemithorax (Space-filling) with Pulmonary Resection

Use of thoracoplasty prior to resection in 39 hospitals

Not used or rarely used	20
Occasionally used	7
Moderate to frequent use	12

The chief reason given for the use of thoracoplasty prior to resection is the anticipation of space-filling difficulty at the time of resection in poor-risk patients in whom concomi-

[1] Presented in part before the Sixteenth Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis, St. Louis, Missouri, February 12, 1957.

[2] These forty-four hospitals were: the Fitzsimons Army Hospital, Denver, Colorado; the Parks Air Force Base Hospital, California; the U.S. Naval Hospital, St. Albans, New York; and the following Veterans Administration hospitals and centers: Albany, New York; Albuquerque, New Mexico; Alexandria, Louisiana; Augusta, Georgia; Baltimore, Maryland; Batavia, New York; Brooklyn, New York; Bronx, New York; Butler, Pennsylvania; Castle Point, New York; Coral Gables, Florida; Dayton, Ohio; Dearborn, Michigan; Downey, Illinois; Durham, North Carolina; East Orange, New Jersey; Hines (Chicago), Illinois; Indianapolis (Cold Spring Road), Indiana; Iowa City, Iowa; Kerrville, Texas; Livermore, California; Louisville, Kentucky; McKinney (Dallas), Texas; Madison, Wisconsin; Martinsburg, West Virginia; Memphis (Kennedy Medical Teaching Group Hospital), Tennessee; Minneapolis, Minnesota; New Orleans, Louisiana; Oteen, North Carolina; Portland, Oregon; Rutland Heights, Massachusetts; Salt Lake City (Fort Douglas), Utah; San Fernando, California; Sunmount, New York; Temple, Texas; Tucson, Arizona; Walla Walla, Washington; Waukesha, Wisconsin; West Haven, Connecticut; Whipple, Arizona; and Wood (Milwaukee), Wisconsin.

tant thoracoplasty would add appreciably to the operative risk. Another reason is the belief that resection is better tolerated in some poor-risk cases after a thoracoplasty.

One hospital reported the use of prior thoracoplasty for pneumonectomy only.

USE OF THORACOPLASTY CONCOMITANT WITH RESECTION IN 38 HOSPITALS

Not used or rarely used	12
Occasionally used	9
Moderate to frequent use	17

In general, those surgeons using prior and concomitant thoracoplasty believe that the best way to prevent bronchopleural fistulas, empyemas, and residual air spaces is to be sure that the remaining lung tissue fills the pleural cavity promptly following resection, and that subsequent thoracoplasty cannot be performed soon enough after the resection in order to accomplish this in all cases.

Seven of the twelve surgeons not favoring concomitant thoracoplasty are partial to prior thoracoplasty, thus leaving only five hospitals where either prior or concomitant thoracoplasties are not used.

USE OF THORACOPLASTY SUBSEQUENT TO RESECTION IN 29 HOSPITALS

Used for complications only	12
Used electively:	
Occasional use	2
Moderate to frequent use	14
After pneumonectomy:	
Used routinely	6
(Others use when indicated)	

One of the six hospitals using thoracoplasty routinely after pneumonectomy does so only when the patient is less than forty-five years of age.

USE OF SPACE-FILLING PROCEDURES OTHER THAN THORACOPLASTY WITH RESECTION IN 15 HOSPITALS

Preresection:	
Extraperiosteal plombage	2
Routine pneumoperitoneum	1
Concomitant:	
Pleural tents	3
Extraperiosteal stripping	3
Phrenic paralysis	3
Extraperiosteal plombage	1
Pneumoperitoneum	1
Postresection:	
Pneumoperitoneum	2

Only a few hospitals exclude the use of thoracoplasty entirely for one or more of these other procedures.

The Use of a Previously Unused Antituberculosis Drug to Cover Resectional Surgery in Patients with Cavities and/or Bacillary Resistance

USE OF ADDED DRUG COVERAGE FOR RESECTION IN 41 HOSPITALS

Only in resistant cases	18
Resistant and/or cavitary	10

Resistant and/or "positive"	3
Cavitary and/or "positive"	3
Special indications	3
After long-term chemotherapy	2
Only with endobronchial disease	1
All resections	1

In general, there is a tendency to be on the safe side by adding a previously unused drug to cover resectional surgery for reasons other than laboratory evidence of bacillary resistance; a few cautious surgeons use added drug coverage in practically every case.

The Choice of Resection Procedures

PREFERENCE FOR TYPE OF RESECTION

Use of segmental and subsegmental resections	
No prejudice against the use of either	34
Do not use subsegmental resections or have reservations	6
Type of resection in resistant cases	
Lobectomies preferred to smaller resections	16
Use segmental resection	9

The question as to the use of small resections was asked because it was realized that some surgeons believe that the bronchus is frequently involved by the tuberculous process and is divided too close to the lesion when a subsegmental resection is performed.

The second part of this question asked whether a lobectomy rather than a smaller resection should be performed in patients having resistant organisms. The answers indicated that there is a definite tendency not to use segmental and subsegmental resections in resistant cases, and even a tendency to prefer the resection of whole lobes in all patients with cavitation and/or sputum containing tubercle bacilli.

Indications for Resection of "Closed Lesions"

INDICATIONS FOR RESECTION OF CLOSED LESIONS IN 38 HOSPITALS

Special indications (youth, relapse, et cetera)	15
3.0 cm. or over	1
2.5 cm. or over	1
2.0 cm. or over	10
1.5 cm. or over	1
1.0 cm. or over	4
Cavities filling on chemotherapy	4
Do not resect	1
"When seen by X-ray"	1

There were many qualifications in the answers to this question. This tabulation is, therefore, a rough one. Even though a number of the hospitals indicated a particular lesion size, their replies implied that the resection of closed lesions is recommended on an individual basis for the most part.

The contrast between the replies listed in the last two lines is marked; all closed lesions are resected in one hospital, while none are resected in another.

The Use of Collapse Therapy when Resection is not Feasible or when the Risk of Resection is High

USE OF COLLAPSE THERAPY IN 42 HOSPITALS

Not used	4
Rarely used	4

Used; type not specified	7
Thoracoplasty only	10
Extraperiosteal plombage only	10
Thoracoplasty and extraperiosteal plombage	7

Thoracoplasty and/or extraperiosteal plombage are used in a majority of hospitals. The indications include situations which render resection either not feasible or excessively risky, such as extensive disease, bacillary resistance, and so forth.

Four hospitals reported that they had abandoned the use of extraperiosteal plombage because of a high infection rate in the extraperiosteal space.

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April 29, 1957

LYMPHATIC DRAINAGE OF THE PERICARDIAL SPACE IN DOGS, AS DETERMINED BY STUDIES WITH RADIOACTIVE GOLD (Au^{198})

The relationship between tuberculous pericarditis and caseous tuberculosis of mediastinal lymph nodes has long been recognized.[1] Direct extension of disease from these lymph nodes to the immediately adjacent pericardium is accepted as the possible etiology of tuberculous pericarditis in the majority of cases.[2] The mediastinal nodes may become involved secondarily to tuberculosis of the lung or pleural space.[3]

It may be possible, however, that in certain instances the tuberculous pericarditis occurs first, followed by involvement of the mediastinal lymph nodes by virtue of lymphatic drainage from the pericardium. There is clinical support for this concept in the findings of cases of apparently "primary" tuberculous pericarditis[4] and in the rare case of involvement of the pericardium and mediastinal nodes without there being any tubercles in the lung or elsewhere in the body.[5]

These writers are now studying lymphatic drainage to and from the pericardium in dogs, using radiogold (Au^{198}) as a tracer substance. From this study, information has been obtained that in the dog the lymphatic drainage from the pericardium, a subject that is poorly documented anatomically, is primarily to the mediastinal and pulmonary hilar lymph nodes. In addition to its value in studying pathogenic processes in tuberculosis, this information also may be of clinical interest in respect to the removal, via lymphatics, of various transudates and exudates from the pericardial space.

Adult mongrel dogs were anesthetized by sodium pentobarbital and were given positive pressure endotracheal oxygen. Under sterile procedure, a small anterior fourth interspace thoracotomy was done. Using a small polyethylene tube inserted through a nick in the pericardium, a tracer dose (approximately 2 millicuries) of radiogold colloid (Aurcoloid®)[6] was placed in the pericardial space. The polyethylene tube was removed without contamination of the pleura, the hole in the pericardium was closed by a purse-string suture, and the area reinforced by suturing pericardial fat over it. The lung was expanded and the thoracotomy closed. Forty-eight hours later the animal was sacrificed and a complete autopsy was done, removing tissue specimens as detailed in table 1. The pericardium was entered as the last step of autopsy in order to minimize contamination. Tissue specimens were rinsed three times in saline, blotted, weighed wet in a Roller-Smith balance, and radioactivity measurements were made in a scintillation well-counter.

High counts were of course obtained from pericardial and myocardial tissues which were directly exposed to the radiogold. Substernal nodes, when present, usually adjacent to the first part of the internal mammary artery, showed elevated counts. The important finding, however, was the consistently high count present in the bronchial, subcarinal, and paratracheal groups of mediastinal nodes. It can be concluded that in the dog lymphatic drainage of intrapericardial particulate matter of this size is primarily to the mediastinal lymph nodes.

[1] Weigert, C.: Deutsch. med. Wchnschr., 1883, *9*.
[2] Osler, W.: Am. J. M. Sc., 1893, *105*, 20.
[3] Perkins, R. B., *et al.*: Am. Rev. Tuberc., 1957, *75*, 145.
[4] Reisman, D.: Am. J. M. Sc., 1901, *122*, 6.
[5] Abbott Laboratories. Aurcoloid is radiogold (Au^{198}) colloid, the particles being approximately 0.003 u in diameter. Half-life is 2.69 days, and the radiation consists primarily of a 0.97 mev beta ray and a 0.411 mev gamma component.

TABLE 1

TISSUE CONCENTRATIONS OF RADIOGOLD IN VARIOUS TISSUES 48 HOURS AFTER INTRAPERICARDIAL ADMINISTRATION OF Au^{198}

Tissue	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8
Left axillary node	348	101	1,180	3,060	1,120	622	5,250	5,050
Right axillary node	367	598	856	900	934	473	2,780	2,950
Left cervical node	A*	541	630	492 611	A	A	3,960	13,200
Right cervical node	A	626	547	485	433	479	1,570	12,400
Splenic node	517	9,270	736	3,940,000	57,500,000	19,500	1,360	3,120
Hepatic node	5,230	2,320	1,170	1,060	26,100	2,410	8,250	1,400
Left lumbar node	299	920	394	1,760	7,310	464	3,050	212,000
Right lumbar node	541	16,400	318	815	634,000	472	1,500	1,770
Left main bronchus node	455,000	1,360,000	8,460,000	13,400,000	36,000,000	550,000	265,000	A
Right main bronchus node	2,170,000	8,080,000	152,000	437,000 8,490	3,270,000	13,200,000	9,790,000	14,300,000
Subcarinal node	535,000	26,000	424,000 477,000	242,000	7,070,000 14,800,000	1,080,000 7,610	94,700	5,550,000
Left paratracheal node	9,520,000	46,700,000	22,400,000	18,100,000	229,000,000	149,000	160,000	5,880
Right paratracheal node	612,000	4,400,000	28,600,000	1,770,000	A	3,540,000	7,340,000	4,160
Left substernal node	A	A	A	A	A	809,000	A	782,000
Right substernal node	A	A	300,000	9,950,000	11,500,000	7,460,000	6,650,000	24,400,000
Esophageal node	A	A	A	A	A	A	A	3,430,000
Left diaphragm	3,040	5,580	5,070	11,200	15,400	811,000	3,900	10,700
Right diaphragm	1,080	4,370	2,790	8,400	2,280	7,940	47,200	16,400
Left lung	6,130	15,600	28,100	335,000	13,300	684,000	58,200	6,110,000
Right lung	1,790	3,500	1,240	2,050	6,280	12,700	8,300	1,570,000
Trachea	3,390	7,730	3,430	7,670	39,700	3,660	3,980	1,940
Pericardium	1,310,000	77,500,000	9,180,000	5,180,000	3,070,000	4,650,000	8,720,000	47,600
Myocardium	74,500	333,000	40,500	39,100	195,000	714,000	179,000	40,900,000

* A = Tissue absent in that animal.

Results are expressed as counts per minute per gram of tissue, correction being made for isotope decay. In some instances more than one lymph node was found in the given location, and these were individually counted.

In summary: Radiogold placed in the pericardial space of dogs is taken up primarily by mediastinal lymph nodes. The possible clinical significance of this lymphatic drainage route is mentioned.

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May 29, 1957

STUDIES ON STERILIZATION OF CYSTOSCOPES

Effective and safe sterilization of cystoscopes is an important problem for all urologists. The variety of organisms which must be overcome is so great that most of the sterilizing agents available are incapable of killing all forms of bacteria in a reasonable time. Protection of the cement which holds the lenses and light bulbs must also be considered. The ideal agent also should be easy to handle and not be irritating to mucous membranes or to the skin.

It is the practice of most hospitals to employ solutions of oxycyanide, 1 to 1,000, or of quaternary ammonium compounds in an effort to control the usual organisms from the genitourinary tract. While these agents may control some organisms, they do not kill *M. tuberculosis*.

The present writers have previously shown that a 10 per cent solution of formaldehyde will kill *M. tuberculosis* in five minutes in the absence of organic material. The undesirable aspects of this solution is its tendency to cause dermatitis of the hands among nursing personnel who handle the instruments. Fitzsimons Army Hospital in Denver, Colorado had reported encouraging results with Wescodyne®,[1] an iodophor preparation. Briefly, an iodophor is a combination of iodine and a surfactant. The Lehn & Fink Co. have offered Amphyl®,[2] Osyl®[3] and L & F Instrument Germicide®,[4] Forma-san®,[5] another germicide, has also been advocated as a tuberculocidal solution for cold sterilization of cystoscopes.

To test the merits of these various compounds, the following studies were undertaken. Cystoscopes were inoculated with a wet weight of pathogenic *M. tuberculosis*, 0.5 mg. to 1.5 mg. per ml. of diluent. These organisms had been recovered from patients with renal tuberculosis. Representative growth from three different strains of freshly cultivated *M. tuberculosis* was included in this test material. After rinsing the contaminated instruments in running tap water for five seconds, the respective germicides were added to sterilized dishes so as to completely immerse each cystoscope for the specified time. The instrument was then removed, washed in running tap water for five seconds, and returned to the container, which had also been washed with water. Cultures were taken and checked for growth at weekly intervals for eight weeks.

The first study was a recheck of our original work. In table 1 it may be seen that again 10 per cent formaldehyde was uniformly effective with both serum and saline inoculations of *M. tuberculosis*. After thirty minutes of immersion, 1 per cent formaldehyde was not effective. The 5 per cent solution did offer satisfactory sterilization in fifteen minutes.

That Forma-san formula was uniformly effective in five minutes is shown in table 2.

[1] Polyethoxy polypropoxy ethanol iodine complex, 7.75 per cent; nonyl phenyl ether of polyethylene glycoliodine complex, 7.75 per cent; hydrogen chloride (provides 1.6 per cent available iodine), 0.10 per cent; inert ingredients, 84.40 per cent.

[2] Potassium ricinoleate, o-phenylphenol, p-tert amyphenol and alcohol (active ingredients), 70 per cent; water, propylene glycol and glycerol (inactive ingredients), 30 per cent.

[3] Potassium ricinoleate, orthohydroxydiphenyl and alcohol (active ingredients), 45 per cent; water, propylene glycol, glycerol (inactive ingredients), 55 per cent.

[4] Potassium ricinoleate, o-hydroxydiphenyl and p-tert amylphenol (active ingredients), 4.75 per cent; water, propylene glycol, sodium metasilicate (inactive ingredients), 95.25 per cent.

[5] Isopropanol; methanol; formaldehyde, 3.5 per cent; benzalkonium chloride.

TABLE 1

RESULTS OF TESTS WITH FORMALDEHYDE

Immersion Time	5 Minutes	10 Minutes	15 Minutes	30 Minutes
10% Formaldehyde	No growth	No growth	—	—
5% Formaldehyde	Growth	Growth	No growth	—
1% Formaldehyde	—	Growth	Growth	Growth

TABLE 2

COMPARISON OF TESTS WITH VARIOUS STERILIZING DRUGS OTHER THAN FORMALDEHYDE

Immersion Time	5 Minutes	10 Minutes	30 Minutes	60 Minutes
Forma-san	No growth	No growth	—	—
L & F Instrument Germicide	Growth	Growth	No growth	No growth
Osyl 1%	Growth	Growth	No growth	No growth
Amphyl 1%	Growth	Growth	Growth	No growth

TABLE 3

COMPARISON OF WESCODYNE WITH OTHER STERILIZING DRUGS

Immersion Time	5 Minutes	15 Minutes	30 Minutes
Wescodyne	Growth	Growth	No growth
Osyl 2%	Growth	Growth	No growth
Amphyl 2%	Growth	Growth	No growth

When repeated three months later it again showed the same good results. The results with Osyl and Amphyl formulas showed them to be unsatisfactory. L & F Instrument Germicide was found to be effective after thirty and sixty minutes. For the shorter intervals it was not consistently effective. One inference was that perhaps some strains of *M. tuberculosis* were resistant to this germicide.

As may be seen in table 3, the Wescodyne formula was not effective in fifteen minutes but did produce consistent sterilization in thirty minutes. This was disappointing since the detergent action of this solution is high and would be a desirable feature.

All of the solutions mentioned except formaldehyde have been shown by experience to be nonirritating to the mucous membrane. It should again be emphasized that the instruments should be placed in oxycyanide solution or a similar agent to wash off the excess formaldehyde.

In summary: Controlled studies have been done during the past eighteen months testing various solutions for their ability to sterilize cystoscopes against *M. tuberculosis*. Immersion in 10 per cent formaldehyde for five minutes was again proved to be satisfactory. One other solution, Forma-san was also found to be dependable in five minutes. Other solutions tested, i.e., 1 and 5 per cent formaldehyde, Osyl, Amphyl, L & F Instrument Germicide, and Wescodyne did not give consistent results and/or required much longer times to accomplish satisfactory sterilization.

RESEARCH UNIT FOR GENITO-URINARY TUBERCULOSIS
BRONX VETERANS ADMINISTRATION HOSPITAL
BRONX, NEW YORK

HERMAN WECHSLER[6]
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GEORGE ROSENBLATT
MILTON GOLDMAN

June 3, 1957

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(1) LATTIMER, JOHN K., *et al.*: J. Urol., 1956, *76*, no. 2.
(2) JAMES A. WIER, personal communication.

[6] The writers wish to offer their thanks to the following manufacturers for their generous supply of testing material: Huntington Laboratories, Inc. of Huntington, Indiana, for Forma-san; Lehn & Fink Products Corp. of New York City for Amphyl, L & F Instrument Germicide and Osyl; and West Disinfecting Co. of New York City for Wescodyne.

They are also grateful to the American Cystoscope Makers, Inc., for testing Forma-san on the lens cement and conducting systems of the cystoscopes.

Correspondence

PERSONNEL PRESSURE AND THE TUBERCULOSIS PATIENT

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

In a moment of reverie not too long ago, the writer found himself preoccupied with the following "interesting case." With the thought that the lessons derived from this case might have some practical application, the case record is presented below.

A patient with tuberculosis visited a Mass X-ray Fluorographic Unit. He had been led to attend by a pamphlet prepared by a tuberculosis health educator and distributed by the public relations director of the local tuberculosis association, as well as by the urging of the local health officer and his department's tuberculosis control division, conveyed through their publicity service, and the daily newspaper, radio, and television.

A minifilm was taken at the Unit by a technician after the patient's name and identifying information had been recorded by a clerk. Later he received a card from the secretary requesting him to return for a large film because of an abnormal shadow in the minifilm. After another letter, a telephone call, and a visit from the Health Department visiting nurse, he went to a recheck center. There he gave his epidemiologic history to a medical social worker, partially disrobed as directed by the station nurse, had the film taken by the technician, and recovered his clothes from the attendant. Then he received a letter from the secretary referring him to his private physician, who had been informed of the result of the X-ray. He called up the physician, and made an appointment with the office secretary. At the appointed time the receptionist filled out his financial record, the nurse took his temperature and a sputum specimen, and the physician asked further questions, examined his chest, and told him that he had tuberculosis and advised him to enter a sanatorium. As he was unable to pay for prolonged institutional care he was referred at once to the Health Department and told to go to the clinic there. At the clinic he was again checked by the clerk and interviewed by a medical social worker regarding his finances, and by the clinic physician who again looked at his films, listened to his chest, and forwarded the application for admission to the sanatorium.

He returned home to await admission, and a relief social worker came to the home to help make arrangements, to obtain financial aid for his family, and to help in other adjustments. A visiting nurse came to explain to the patient and his family the facts about tuberculosis and the need for precautions, and a health department inspector visited to see that they were not being neglected.

Then an ambulance driver and attendant took him to a ward. The ward nurse made him comfortable, an attendant put away his clothes, and an orderly brought him a drink. During the first day he was welcomed by the head nurse and chief nurse, and attended by several other ward nurses and the night nurse. He was fed by the maids and waitresses after diet had been checked by the dietician. His radio-television setup was adjusted by the radio mechanic, and the floor was swept by the janitor. He was interviewed and examined by the ward physician, who dictated his findings to the medical secretary, and a record clerk took a social history. Later the Chief Medical Director and his assistant, the Chief Tuberculosis Physician, a sanatorium social worker, a chaplain, a psychometrist, a clinical psychologist, a psychiatrist, a vocational counselor, a physical therapist, an occupational therapist, an adult education worker, and a librarian visited him.

During the following weeks a vocational trainer, a recreational worker, a rehabilita-

tion aide, representatives of the special services of the American Legion and the Veterans of Foreign Wars, and several church group volunteers also called. He was transferred to a treatment ward by the institution ambulance driver and orderly, where he met a new ward physician and was referred to the otolaryngologist, the dentist, the radiologist, the pulmonary function physiologist, the different laboratory technicians who took blood specimens, gastric lavage, vital capacity and electrocardiograms, the pathologist, the cardiologist, and the orthopedic consultant. However, the patient did not have contact with the chest surgeon and all of the staff of the surgical service, as had many others. Nevertheless, he did receive medication brought by the messenger from the pharmacist and administered by a special treatment nurse in charge of injections. His family were contacted by the visiting nurse and asked to go to the Red Cross blood bank to leave blood for him should it ever be required, and there they met nurses and technicians who operated the blood bank.

The patient met other patients, his bed mates and the others in his wards, and also ambulatory patients from other wards, and the members of patient groups, not in Alcoholics Anonymous or group psychotherapy classes, but the staff of the patient paper, entertainment groups, et cetera. Visitors, members of his family, friends, and strangers coming to see other patients or member of organizations stopped at his bedside. Besides the patients' paper he read pamphlets and books brought him by the librarian and contact officers, and letters from his own family and friends and from the tuberculosis association and advertisers. He heard the radio, and the wired sanatorium broadcasts using the same receivers; he saw the television programs and, towards the end of his stay, he met the occupational placement worker, technical teachers, rehabilitation physician, and liaison social workers.

Even after discharge he still maintained his contact with the outpatient clinic physician and staff, the nursing and social workers, the health department inspector, the follow-up workers, and others.

The patient was somewhat confused by the large number of different strangers he encountered. He sometimes failed to recognize them at different times, or to remember just what he had told each one before. Not only did he meet so many different workers, but to some of them he seemed to be just one of a large number of patients with whom they were concerned, and so they had time and interest for only one phase of his care. Even his physician changed repeatedly as he progressed through the various locations, in addition to changes due to rotation and other changes in institutional assignments. It was difficult for him to develop a feeling of intimacy and reliance on any one individual, to know to just whom he should divulge various complaints or desires, and whose advice he should seek and follow on various medical and nonmedical matters.

The advantages of the elaborate organization of the medical and paramedical facilities were apparent. Every one of the hundred or more persons whom he met had been especially trained, was well qualified to give him some special service, and was eager and able to devote the time needed to do so. He certainly received benefit from some, and possibly from all of them. Special efforts had been made to overcome some of the defects inherent in the system. Group conferences were held at which various types of workers presented different aspects of the case, and the various charts and records which they kept made problems and possibilities which had been revealed to some of them available to the others. Team-work and communication, especially important because of their multiplicity, were facilitated by the special provisions made for the coordination and correlation of their efforts. Whenever possible, everything related to the patient was

channeled through his attending physician, and a deliberate attempt was made to foster the personal patient-doctor relationship despite the maze of specialized services.

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EMIL BOGEN

April 5, 1957

COMMINUTION OF MYCOBACTERIA BY EXPOSURE TO ULTRASONICS

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

In order to achieve suspensions of tubercle bacilli consisting mainly of a single bacterium, the number of bacterial clumps are generally reduced by means of filtration or centrifugation.

The writer has investigated the possibility of comminuting the bacterial clumps, without influencing the viability of the bacilli, by means of ultrasonic exposure of the bacterial suspensions.

The ultrasonic emitter used in the experiments is a Siemens Sonostat Standard, which sends out ultrasonic waves with a frequency of 800 kHz. The experimental apparatus employed ensures ultrasonic exposure of the bacterial suspensions at intensities varying from 0.05 watt/cm.^{2} to 0.56 watt/cm.^{2}, measured in the exposed suspensions.

The bacterial suspensions (6-day-old BCG cultures grown in Dubos fluid medium with Tween®) are rendered homogeneous macroscopically by shaking, and are then exposed to ultrasonic treatment in a flat-bottomed aluminium container at five different intensities for periods of up to twenty-six hours.

The effect is judged by (*1*) counting the number of bacteria in the bacterial units on slides stained by the Ziehl-Neelsen method, and (*2*) by culture of different dilutions of the exposed bacterial suspensions.

Ultrasonic exposure for a few minutes at the lowest intensity (0.05 watt/cm.^{2}) results in a reduction in the number of single bacteria and an increase in the number of clumps of more than 5 bacteria counted in the smears. This "agglutinating" effect is confirmed by estimation of the number of viable bacilli by culture; this shows a reduction in the number of bacterial units per ml. of suspension.

Ultrasonic exposure at higher intensities shows a greater dispersing effect with a more rapid onset the higher the intensity used.

While the non-sonic treated bacterial suspension contains 22 per cent single bacterium and 50 per cent clumps of more than 5 bacteria, ultrasonic exposure for five to ten minutes at the highest intensity (0.56 watt/cm.^{2}) results in suspensions with more than 80 per cent single bacterium and about 10 per cent clumps of 2 bacteria; the number of clumps containing more than 5 bacteria is less than 1 per cent. Correspondingly, the increase in the number of viable bacterial units is ten times higher than the control values.

Ultrasonic exposure for longer periods at an intensity of 0.56 watt/cm.^{2} results in destruction of bacteria, the extent of which shows a linear dependency between the logarithm of the number of surviving bacterial units and the length of the ultrasonic exposure. After twenty-six hours' exposure 15 per cent of the bacterial units are still viable. Since the maximal dispersing effect is achieved after only five to ten minutes' exposure, the bactericidal effect at that time will be negligible.

Other experiments have been carried out with the same technique in which 5 strains of mycobacteria (tubercle bacilli of human type, bovine type, avian type, and BCG, and a chromogenic saprophyte) have been exposed to ultrasonics at an intensity of 0.56 watt/cm².

These experiments showed a dispersing effect similar to that mentioned above, and also a similar bactericidal effect.

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BENT MACKEPRANG

June 7, 1957

THE CHEMOTHERAPEUTIC ACTIVITY OF TRITON WR 1339 AND MACROCYCLON IN MURINE LEPROSY

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Kátó and Gözsy,[1] in a recent note to the REVIEW, compared the therapeutic activity of 1,4-dimethyl-7-isopropyl-bicyclo-decapentane with the surface-active agent, Triton A-20, in experimental tuberculosis and leprosy. Their results confirmed the powerful antituberculous activity of Triton A-20 (a 25 per cent aqueous solution of the solute Triton WR 1339) in the mouse and guinea pig reported earlier by ourselves[2, 3] and others.[4] In murine leprosy, on the other hand, they failed to show therapeutic activity with either compound in the rat or the mouse while we have found, in hitherto unpublished work, that the Triton type of surface-active agents are active against experimental leprosy in the mouse. A brief report is therefore given of the therapeutic tests on which the favorable results are based.

Mice were infected intravenously with a partially purified suspension of *M. lepraemurium* obtained from homogenized rat lepromata. This method of infection produces a reasonably standard type of systemic infection involving particularly the liver, spleen, skin, and heart. The progress of the infection was assessed by macro- and microscopic examination of the various tissues and by carrying out bacillary counts (of stainable acid-fast bacilli) on homogenates from the liver and spleen. The surface-active agents were administered subcutaneously, twice weekly, commencing on the day after infection. With this standardized mouse test, Triton WR 1339 and Macrocyclon (a less toxic though chemically similar type of surface-active agent with a macrocyclic structure, referred to by Cornforth[5] by the code number GN/57) administered in doses of 5 mg. for twenty to twenty-seven weeks, showed high therapeutic activity.

Kátó and Gözsy[1] failed to show any activity with Triton A-20 against an infection with murine leprosy in either the rat or the mouse. Although they used larger doses of drug (10 mg. daily) than in the present study, the results were assessed after only six to eight weeks of treatment in animals which had been infected either by the subcutaneous (in rats) or intraperitoneal (in mice) route. While it is not possible to exclude the different routes of infection as the basis for these apparently contradictory results, it would seem

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much more likely that the beneficial effect observed in the present work was due to the longer course of treatment.

Hitherto the therapeutic activity of surface-active agents of the Triton type had proved to be remarkably specific for *M. tuberculosis*, but the result of the present study shows that it undoubtedly extends to other species of Mycobacteria. Furthermore, the results of the present study strengthen the suggestion, put forward by Mackaness[6] and Lovelock and Rees,[7] that the monocyte is the site of action of these agents *in vivo*, since murine leprosy is caused by an obligate intracellular organism infecting cells of the reticuloendothelial system.

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July 6, 1957

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Books

CAUSAL FACTORS IN CANCER OF THE LUNG. By Carl V. Weller. Pp. 113, Charles C Thomas, Springfield, Illinois, 1955, Price, $3.00.

This monograph fills a long apparent void in all of the vast amount of literature that has accumulated in recent years on the subject of causal factors in lung cancer. Here is a work which is a review of a man's inquiry into and understanding of etiologic factors in pulmonary carcinoma. An expanded version of the Beaumont lecture, it is at the same time comprehensive, scholarly, and above all objective and unemotional. The author adduces no new data. His contribution lies in the lucid manner in which he reviews the data already at hand and reaches his own conclusions.

Dr. Weller can be accused of slighting no one. He starts back at least five hundred years with the lung disease, now known to be cancer, which exacted such a heavy toll among the generations of miners in Schneeberg and Joachimasthal. A summary follows of the evidence relating to the relative roles of chalicosis and exposure to cobalt, nickel, bismuth, arsenic, and ionizing radiation in the causation of this endemic occupational disease.

Next the author traces the spectacular absolute increase in the incidence in lung cancer in the twentieth century, and carefully considers the possible role of environmental factors more or less peculiar to those areas and population groups where the rise has been most marked. He dismisses infective pneumonitis and the influenza pandemic as etiologic factors. The increased liability to pulmonary cancer in certain occupational groups is recognized, but the writer quite properly points out that such a correlation, however valid, could account for only a minute fraction of the over-all increase.

Dr. Weller then considers the possible roles played by atmospheric pollution, including its most suspect cardinogenic constituents, arsenic, ionizing radiation, and benzpyrene.

Finally, the author reviews the prospective and retrospective epidemiologic evidence and the laboratory data linking cigarette smoking and bronchogenic carcinoma. He concedes that . . . "I have been resistant to a ready acceptance of the association between cigarette smoking and bronchogenic carcinoma. I have searched the literature for other reasonable explanations or for recognizable fallacies. I have found none of importance." Most commendably, he concludes by emphasizing the fact that four-fifths of the deaths from bronchogenic carcinoma today are potentially avoidable and expresses the hope that the search for practical preventive measures will push ahead.

Such a clear and dispassionate review of the evidence by a man whose interest in the subject extends back over three decades is a most welcome addition to the literature on the etiology of lung cancer.

WALTER E. O'DONNELL
New York, New York

INTERNAL MEDICINE. A Physiologic and Clinical Approach to Disease. Pp. 706, Year Book Publishers, Inc., Chicago, 1956. Price $10.00.

This relatively brief review of internal medicine lives up to its subtitle. In its 685 pages of text the major subdivisions of internal medicine are considered under the following headings: Disorders of the Heart, Hypertension and Vascular Disease, Renal Disease and Renal Failure, Body Fluids and Electrolytes, Endocrine Disorders, Infectious Diseases, Pulmonary Diseases, Allergic Diseases, Disorders of the Gastrointestinal Tract, Disorders of the Blood and Blood Forming Organs, Rheumatic Disorders and Diseases of Bone, and Nutritional and Metabolic Disorders. The index of 20 pages is comprehensive and useful.

The author's decision to omit detailed discussion of such highly specialized subjects as electrocardiography and neurology appears justified by the compactness of the volume. He has successfully avoided duplication of the discussion of overlapping subjects, but makes up for any deficiencies in this approach by accurate cross-references within the text.

Recent developments in the physiologic background of diseases are integrated into the review of the pathogenesis, symptoms, signs, and treatment of the diseases considered. In a volume designed for brevity yet retaining a broad scope, this approach is to be admired even though the discussion of purely historical details is slightly curtailed. Equally pleasing is the author's frequent inclusion of pertinent details of bedside procedures or time-tested principles of practical management.

In the treatment of so vast an area of knowledge in a relatively brief text, it is not surprising that some chapters, notably that on Nutritional and Metabolic Disorders, appear to be little more than well-edited catalogues of available information. Generally, this hazard has been skillfully avoided by the author and his able consultants. The sections on renal disease and electrolyte problems are particularly remarkable in their concise and understandable presentation of recent physiologic advances in a frequently confusing area. Late developments in the fields of cardiovascular disease, antimicrobial therapy, endocrinology, and hematology are also handled in an amazingly comprehensive fashion.

This volume makes no claim to be a complete textbook of internal medicine. It does succeed in providing an understandable and accurate review of old and new principles underlying the recognition and management of the major disdisorders encountered in internal medicine. It is to be especially recommended for the specialist, the senior medical student, and any physician who desires an up-to-date review of the topics covered in this book.

WILLIAM N. CHRISTENSON
New York, New York

BOOKS RECEIVED

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TUBERCULOSIS NURSING. Ed. 2. By Jessie G. Eyre. Pp. 354, H. K. Lewis & Co. Ltd., London, 1957, Price, £1.5s.

THE SURGICAL MANAGEMENT OF PULMONARY TUBERCULOSIS. No. 1. The John Alexander Monograph Series, Edited by John D. Steele, Pp. 213, Charles C Thomas, Springfield, 1957.

REFERÁTOVÝ V ÝBĚR TUBERKULOSY A PNEMOLOGIE. By Erich Jancik. pp. 182

CHARLES J. HATFIELD LECTURE

The following is the introduction by Dr. Esmond R. Long of Professor Arvid Wallgren, of Stockholm, Sweden, on the occasion of his delivery of the Hatfield Lecture. Dr. Wallgren's lecture is presented on pages 715 to 725 of this issue.

The Charles J. Hatfield Lecture, which will be delivered today, is sponsored by the Potts Memorial Institute, a now venerable institution founded in the mid-nineteen twenties under the terms of the will of Miss Ida Potts of Livingston, New York, which provided for the establishment of a tuberculosis hospital in Columbia County of that state and for the creation of a board of trustees for its management. At the guiding suggestion of Dr. John T. Edwards, an original member and now president of the board, who is with us today, the legal authorities entrusted with execution of the directions of the will sought the advice of the National Tuberculosis Association on the development of the institution. It was decided that the new institution could serve a highly useful purpose in a relatively undeveloped field in tuberculosis control in this country, viz., rehabilitation. With the counsel of the NTA the institution was organized near Hudson New York, under the direction of Dr. Harry Pattison, formerly of the NTA staff, who held this position with efficiency until his death a few months ago. Under his guidance and that of a devoted group of trustees, a sanatorium was organized with an associated print shop and other activities, where convalescent patients and patients with arrested disease could work productively and safely. This sanatorium, like many others in this country, was closed recently, and its associated rehabilitation activities were discontinued. The Potts Memorial Institute has now entered new fields, chief among which are the fostering of research and the advancement of knowledge through media such as this memorial lecture.

Charles J. Hatfield was known to many, perhaps to most of you here. He was president of the Potts Memorial Institute from the time of its founding to the date of his retirement in 1948, a period of twenty-two years. More than fifty years ago he was one of the most active members in that now fast-dwindling group that founded the National Tuberculosis Association. In later years he served as its managing director, its president and for a long term of years, as its secretary. He was highly influential in developing the present form and organization of the National Association, which recognized his contributions in 1937 by award of the Trudeau Medal.

In his home town of Philadelphia he was one of the pioneers associated with Dr. Lawrence F. Flick in the founding of the Henry Phipps Institute for the Study, Treatment and Prevention of Tuberculosis, another institution which, over the years, has been closely connected with the National Tuberculosis Association. On Dr. Flick's resignation from the Phipps Institute in 1909, Dr. Hatfield succeeded him as executive director, a position he retained, with occasional changes in title, for nearly forty years. During all of that long period he was intimately concerned with the antituberculosis activities of Philadelphia. He was a member of the directing boards of the Philadelphia and Pennsylvania affiliates of the National Tuberculosis Association, and served as president of the Philadelphia organization for the long period from 1923 to 1947. He was active in

many other educational and philanthropic enterprises in Philadelphia, a life trustee of the University of Pennsylvania, and a long-time guiding force in the Health and Welfare Council of that city and Southeastern Branch of the American Red Cross. His death in 1951 at the age of eighty-four, after some years of failing health, was deeply mourned by this association.

The Charles James Hatfield Lecture is being given this afternoon by an outstanding pediatrician and immunologist from Stockholm. Professor Arvid Wallgren is well known to members of the National Tuberculosis Association. He has been with us in this country on numerous occasions, and those of our members who have attended meetings of the International Union Against Tuberculosis have often heard him in the presentation and discussion of papers, and are well acquainted with the man and his work.

Professor Wallgren is a recognized authority on the basic principles underlying the phenomenon of resistance to tuberculosis. He has been a pioneer and long-time worker in the field of BCG vaccination. Of the many addresses he has given on this general subject, one of the best known was his discussion of resistance to tuberculosis at the XIIth Conference of the International Union Against Tuberculosis in Rio de Janeiro in 1952, where he outlined the principles of immunology in tuberculosis in clear and masterly fashion. He correlated effective resistance very closely with native, inborn immunity, pointing out that the best resistance known is that in which good native immunity is enhanced by specific stimulation as, for example, by BCG vaccination. He also pointed out the relative inefficiency of such stimulatory measures in persons deficient in native resistance. Professor Wallgren is a prophet decidedly honored in his own country, as well as in the rest of the world. He was professor of pediatrics and a hospital chief in Gothenburg and Stockholm from 1921 to 1956, becoming emeritus professor in the latter year in the Royal Caroline Medical School of Stockholm. He holds honorary doctorates from universites in Stockholm, Paris, Zurich, Algiers, Wales, Santiago de Chile, and Havana.

He has been associated in the work of innumerable scientific societies in his fields of special interest. He is an experienced editor and a widely travelled and exceedingly popular lecturer. He has written some three hundred scientific articles and several books in the fields of pediatrics, immunology, and tuberculosis.

He is noted for his sound judgment and his logical and unbiased approach in difficult and debated fields. We are fortunate in having him with us today to discuss one of those fields and its problems. Most of us have been beset with uncertainties in respect to artificial immunization against tuberculosis, steering our course as well as we can in the midst of enthusiasms on the one hand and doubts and warnings on the other. We have set the seal of our approval on a limited use of BCG vaccination, but have witheld our decision on the more extensive usage recommended by the warmest advocates of the procedure.

All of us have many questions. We have good reason for satisfaction today in the provision of the Potts Memorial Institute whereby Professor Wallgren, a long-time and honored student of the inherent problems of vaccination, will speak to us under the intriguing title "BCG—Past, Present, and Future."

Recommendations for Use of Vacant Tuberculosis Beds

Statement by the Committee on Administrative Problems

Background: Vacancies—Real or Spurious?

In many parts of the United States, waiting lists for tuberculosis hospitalization have been eliminated and there are an appreciable number of vacancies. The question immediately arises: "What useful purpose can these beds serve when they are no longer needed for tuberculosis care?"

Before attempting to answer this question, it would seem imperative to examine the facts closely to determine whether the presence of vacant tuberculosis beds is a real condition or a spurious development. In a few communities, the vacancies represent a valid, genuine result of the good work of the community in providing sanatorium facilities that have been adequate, both quantitatively and qualitatively. Now the community is obtaining a reward for its efforts; it is ahead of this particular part of the tuberculosis problem, new patients requiring hospital care developing less rapidly than the old ones are being discharged from hospitals. As a result of more effective methods of treatment, available since 1952, the period of sanatorium care has been substantially reduced, thus materially helping the tuberculosis bed situation. In states such as Minnesota, North Dakota, and Washington, where waiting lists were virtually eliminated in 1935, 1938, and 1947, respectively, vacant beds are present in goodly numbers because of the fact that the state has truly provided an adequate number of beds—in short, the vacancies are real!

On the other hand, there are states that list tuberculosis hospital vacancies in which the "empty beds" are spurious for one or more of the following reasons:

1. *Unfortunate distribution of sanatorium beds:* A metropolitan area may have waiting lists for sanatorium care, with vacant beds existing in the more rural areas. To close institutions in the rural areas and to have waiting lists in the urban area is obviously maladministration and poor government, a situation that the state legislature should correct. Patients will travel a distance for tuberculosis hospitalization, *providing* they believe they will receive a high type of modern treatment. In one metropolitan area (Detroit), arrangements are utilized for sending patients to sanatoriums 50 to 100 miles away where vacancies exist in county institutions. Such flexibility is most commendable.

2. *Poor quality of sanatorium care:* Municipal, county, or state tuberculosis hospitals may be substandard, due to factors such as old, dilapidated buildings, inadequate budget, or politically inspired administration. Tuberculous patients will not be referred there by their attending physicians, or the patients themselves will refuse to go to a hospital with a poor reputation.

3. *Patients not advised of sanatorium advantages:* In some centers, it has been observed that a significant proportion of patients with active disease are treated at home without any recommendation for sanatorium care. In one state a study showed that 25 per cent of all the new, active cases reported to the state health department in 1955 were treated at home with no sanatorium care recommended at all, even though there were vacancies existing in three of the four tuberculosis sanatoriums at the same time.

4. *Inadequate case finding:* Community-wide X-ray surveys may not have been conducted thoroughly or abandoned prematurely. Routine roentgenographic examination of general hospital admissions is often neglected. X-ray surveys in jails,

with provision for adequate isolation and treatment of the active cases, may not have been initiated. These and other case-finding methods should be employed systematically before it can be stated that existing beds are not needed.

Therefore, before any plan is devised for the use of vacant tuberculosis hospital beds, the community must investigate its tuberculosis situation closely to determine at the outset whether the vacancies are real or not. If the latter is true, appropriate steps should be taken to correct the deficiencies.

Future Use of Surplus Tuberculosis Hospital Beds

Assuming from the foregoing discussion that the study of the vacant beds situation proves that the surplus is real—then what to do? To operate multiple institutions half full of patients is unsound economically. The per diem costs go up terrifically because of the fixed overhead of each institution. As an example, one state of three million population still has four sanatoriums with less than 100 beds in operation. It has also two large, well-equipped institutions, giving an excellent quality of care, but they have only a little more than 50 per cent occupancy, resulting in a per diem cost which will exceed $20 per day in 1957.

Since 1947 the treatment of tuberculosis has increased in scope and complexity, so that it is quite impossible in small institutions to provide a high type of modern care with the necessary full range of medical, surgical, and ancillary services at a reasonable per diem rate. Qualified professional personnel are usually unavailable or unwilling to be employed in small institutions situated in isolated places. Therefore, in any state the first logical step in reducing the surplus tuberculosis beds would be to discontinue the small institutions with less than 100 beds which cannot provide good, modern care for their patients. Many of these small institutions are of frame construction and so poorly built as to be fire hazards according to modern standards; or they are of such poor design as to be unattractive to patients and inefficient to operate. Some are disadvantageously located for future use as any kind of hospital. They can serve no useful purpose for the care of sick people and should be sold or torn down. Some are capable of conversion at reasonable cost for valuable use as nursing homes or chronic disease hospitals or specialized geriatric institutions. Whether the future operation of these institutions after conversion should be under government administration or private sponsorship depends upon the legal background of the particular state or local jurisdiction and its general philosophy of government.

In addition to planning on a regional basis within a state, there will be instances where vacant tuberculosis hospital beds in one state might be filled by patients from an adjacent state with the necessary contractual arrangement. There are already several instances on record in which such arrangements have been made very successfully; others are currently being considered.

As one example of a conversion problem, surplus beds were present in considerable numbers in the state of Washington in 1952. During 1952–1954 six small sanatoriums, ranging from 55 to 110 beds in size (totaling 410 beds in all), were discontinued as tuberculosis hospitals. It is of interest to note what happened to them: (*a*) The first one (55-bed capacity), discontinued in 1952, was wholly unsuited for future care of sick people, and was converted to a building for demonstration of 4-H projects by the local school board; a useful project and much safer from the standpoint of fire hazard. (*b*) One institution of 60 beds, built along the cottage plan a half century ago, was too inefficient for future use and was simply abandoned, ultimately to be torn down. (*c*) A third institution, built in 1936, of 55 beds, was converted at moderate cost, to be used as a "county in-

firmary" for chronic patients, filling an urgent need in that particular community. (*d*) A fourth institution of 110 beds was converted into a special institution for the care of senile and geriatric patients. This sanatorium with a census of only 36 tuberculous patients in May, 1954, has been remodeled and now has 200 geriatric patients in residence. It is operated as a county-owned, but state-administered institution. (*e*) The fifth and sixth tuberculosis sanatoriums, capacity 64 and 66 beds, respectively, were leased by the counties to private individuals and are now being operated as much needed nursing homes, with 60 and 90 patients, respectively. Experiences comparable to the foregoing can be found in Minnesota and New York. The results of the discontinuance of the small sanatoriums in Washington have been:

1. Better medical care is provided for tuberculous patients in the medium-sized and large institutions with their superior facilities and staff.
2. An appreciable saving of tax funds has been realized.
3. Other types of needed patient care are now being provided.

After the small tuberculosis sanatoriums have been converted to other purposes, consideration must be given to the future use of the medium-sized sanatoriums when they are no longer needed for tuberculosis care. These problems may be similar to those of the small sanatoriums, may be easier of solution, or vastly more difficult—all depending upon the local circumstances. Some of the varied purposes to which medium-sized tuberculosis sanatoriums might be converted are listed as follows:

1. *Modernization for use as general hospitals:* Occasionally this is possible and desirable, particularly when located in a large population center.

2. *Conversion to use as chronic disease hospitals:* If the buildings are of sufficient value to permit their efficient and economical use for fifteen to twenty-five years in the future, such conversions are sound. Almost every community needs an augmentation of this type of facility because of the aging population. Sanatoriums of 100 to 300 beds, located near centers of population, are often well suited to this purpose.

Although it would seem administratively less complicated to convert unused, medium-sized sanatoriums of 100 to 300 beds in entirety to other purposes, the problems of joint usage of a sanatorium for tuberculosis hospitalization and chronic medical care usually are capable of satisfactory, complete solution when there is a genuine cooperative spirit on the part of the participating agencies. Such a joint use can work out very much to the advantage of the community, as has been demonstrated by numerous examples from coast to coast. It must be said that there are also examples of disharmony. The care of tuberculous patients has suffered because of overemphasis on the chronic disease medical care problem, legal restrictions on admission for tuberculosis hospitalization, or personality conflicts between department heads. Also, if the number of tuberculous patients becomes too small, the recruitment of adequate, interested, professional personnel may become exceedingly difficult.

3. *Conversion to rehabilitation centers:* For this very useful purpose, the sanatorium physical plant and equipment should be relatively modern and should also be in, or close to, a metropolitan center to obtain the varied complex consultative services and the many kinds of professional skills necessary for successful operation. The pattern of rehabilitation centers is still evolving. Pioneering still has to be done in most instances.

4. *Miscellaneous uses:* In exceptional circumstances there may be need—and the surplus sanatorium may be found suitable—for conversion to a hospital for the mentally ill, an institution for the care of the mentally retarded, a custodial institution for alcoholics, or some other use appropriate and practical for a particular community.

Comment

There are many states in which patient care improvement as well as a marked saving to the taxpayer could be realized by careful, planned judicious consolidation. In almost every instance, a consolidation to effect these benefits will require an intensive educational campaign. Occasionally a complete overhauling of the administrative arrangement for tuberculosis hospitalization may be necessary.

Equitable agreement by all interested citizens on which institutions should be closed or converted and when this should take place may be difficult to achieve. In one state an official decision to close four regional sanatoriums was disputed in each area. To resolve this conflict the Governor appointed an evaluation team of four capable physicians from outside the state to survey the situation and make recommendations. This use of a team of outside experts may be of help in other states when disagreements arise.

Summary

Before tuberculosis sanatoriums are declared to be "surplus," it is advisable to make sure that the presence of vacant beds genuinely reflects an adequate tuberculosis control program—not a spurious situation caused by poor facilities or poor policies in tuberculosis control.

If surplus beds were really available, the tuberculosis program would be strengthened and improved by a reduction of the surplus. A saving of tax funds and a betterment of patient care will result. Distance of travel for tuberculosis care is not the hardship on patients it was previously thought to be.

Discontinuance should start with the small sanatoriums of 100 beds or less. The buildings might be used for nursing homes, chronic disease hospitals, specialized geriatric institutions, nonmedical purposes, or abandoned.

For those few fortunate states that really have a continuing decrease in beds needed for tuberculosis it will now be necessary to start planning for the discontinuance of the medium-sized sanatoriums, one by one, as the load drops. Other uses suggested for these buildings include conversion to general hospitals, chronic disease hospitals, combination tuberculosis and chronic disease hospitals, and rehabilitation centers.

In many of the forty-eight states, the tuberculosis sanatorium program has grown over the past half century on the basis of expediency of considerations no longer of importance. Generally, it suffered badly from the lack of integration and coherent planning, at least when viewed in the light of present needs. It appears to the committee that a shrinkage of the tuberculosis sanatorium case load is inevitable—the exact timetable of sanatorium discontinuance remains the unanswered question. Therefore, it is suggested that each state study the surplus bed situation (when it truly exists) and prepare an orderly, logical plan for sanatorium discontinuance giving primary consideration to the best interests of patients. This is greatly to be preferred to a haphazard discontinuance of institutions, or parts of institutions, based on political or financial pressures, or other kinds of expediency. The cooperation of the state and local tuberculosis associations, Trudeau societies, medical societies, health departments, and other agencies in the state concerned with tuberculosis control, in working out the best possible program is earnestly recommended. This is essential if patient care is not to suffer.

As a final point, the entire subject of sanatorium discontinuance and the future use of surplus tuberculosis sanatorium facilities is one which should be reviewed with the state agency responsible for hospital planning and construction and the utilization of Hill-

Burton funds. The technical knowledge of the agency staff will be helpful and will assist in preventing mistakes in planning.

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Obituary

Andrew John Moreland

Dr. Andrew John Morland of London died on July 13, 1957. The following remarks were made at a Quaker Memorial Service by Dr. Harley Williams, Secretary-General of the National Association for the Prevention of Tuberculosis.

"I knew Dr. Andrew Morland best as a blend of visionary and practical man. He believed whole-heartedly in exchange between countries. Speaking both French and English, he was very impressive at International Meetings, and how eager he was when it became possible to send British children for treatment in Denmark. Then, he directed a plan for treating other children in Switzerland. He had the same desire for exchanges of knowledge with the Far East.

These ideas he worked out in a manner of his own, inspiring others, ignoring difficulties, calmly proceeding to his objective. He knew by instinct the best way of doing a thing, and I think, this came out of long reflection in those silent moments when he was seemingly wrapped in himself. He was never ambitious to be well thought of, and cared little for being thanked.

I believe our friend was one of those who penetrate further, who feel more, and perceive what is not obvious to most other men.

He radiated the sort of power which every doctor envies. He gave others the confidence which overcomes fear, and helps people to help themselves.

I do not think Andrew Morland had a single enemy in the world. He gained his human ends by methods we should all like to emulate—he achieved them because they were right. He meditated much, and, after he had reached his decision, he let the event bring its own fruit. And generally, he was not disappointed.

How did our friend achieve this unusual wisdom? In this prosaic modern world, he seemed to draw upon mystical resources within himself. He was one of those men who are born wise, but he got inspiration from nature and art. He enjoyed gliding down the snow slopes in the sunshine, but he had first made the arduous upward climb. He loved pictures, and liked also to help those who paint them. With Andrew, silence was a form of communication; he possessed a wordless power which inspired courage and optimism.

In the years of our friendship, I remember no occasion when we differed. Yet, it was not always that we thought the same. Often, our views were opposite. What then, was his secret? It was that his way of looking upon life included so many sympathies. His outlook was free from envy or malice. He had a strange power to unify antagonisms, to reconcile contradictions, to merge thought into action. These are rare gifts, and in Andrew they prevailed, like a natural force.

Having seen these qualities used so well, we ought not to grieve that our friend was taken from us before the normal time. When the hour comes for us to die, we may hope to have the courage he showed when he knew his days were to be short. His family have not only irreplaceable personal memories, they will know that Andrew Morland, in the estimation of the whole world, was a good physician and a good man."

J. H. Harley Williams
London, England

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 19–24
1959—Chicago, Illinois—May 24–29

NTA FELLOWSHIPS

The Medical Section of the National Tuberculosis Association, the American Trudeau Society, provides a limited number of fellowships to promote the training of clinicians, medical teachers and scientific investigators in the field of tuberculosis and respiratory diseases. Awards are open to citizens of the United States for work within this country.

Candidates holding the degrees of M.D., Ph.D. or Sc.D., are eligible for awards making possible continuation of graduate study in the field of respiratory diseases in an approved hospital or medical center. Such studies may be oriented toward teaching or research. Residency in an approved hospital under such a fellowship will be credited by the American Board of Internal Medicine toward certification in internal medicine and pulmonary diseases.

Predoctoral fellowships are also offered to graduate students who hold a bachelor's degree and are working on a research project for an advanced degree other than an M.D.

Each applicant must have the approval of the head of the department under whom he expects to work. All awards are determined by individual circumstances and are paid directly to the Fellow on a quarterly basis. Fellowships are granted for one year. Not more than two renewals will be considered. Fellowship applications must be received by January 1. Appointments may begin on any date at the convenience of the applicant.

TRUDEAU FELLOWSHIPS

A few fellowships at a higher level of training and award are offered to specially qualified candidates with an M.D. degree who have been assured of a continued teaching or research appointment upon completion of training. Trudeau Fellowships are awarded for one year but may be renewed up to a total period of four years.

Further particulars concerning fellowships may be obtained upon request from:

The Director of Medical Education, American Trudeau Society, c/o The Henry Phipps Institute, Seventh and Lombard Streets, Philadelphia 47, Pennsylvania.

COURSE IN LABORATORY METHODS IN THE DIAGNOSIS OF TUBERCULOSIS

In cooperation with the Division of Special Health Services, Public Health Service, the Bacteriology Laboratory of the Communicable Disease Center, Chamblee, Georgia, will offer a course in Laboratory Methods in the Diagnosis of Tuberculosis over the following period: January 20–31, 1958.

This course is open to all grades of employed laboratory personnel who are approved by their State Health Officers. Practical laboratory training in all phases of tuberculosis bacteriology, including preparation of culture media, microscopy, cultural procedures, diagnostic use of animals, and testing of drug sensitivity will be included in the course. Students who attend this course will be offered a Student Extension Service for a period of one year following termination of the course.

Laboratory directors and senior laboratory staff members may also make application for this course.

No tuition or laboratory fees are charged. Reservations for this course should be made well in advance.

Application forms may be obtained from the Laboratory Branch, Communicable Disease Center, Public Health Service, P. O. Box 185, Chamblee, Georgia.

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3rd Annual Postgraduate Course
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December 9–13

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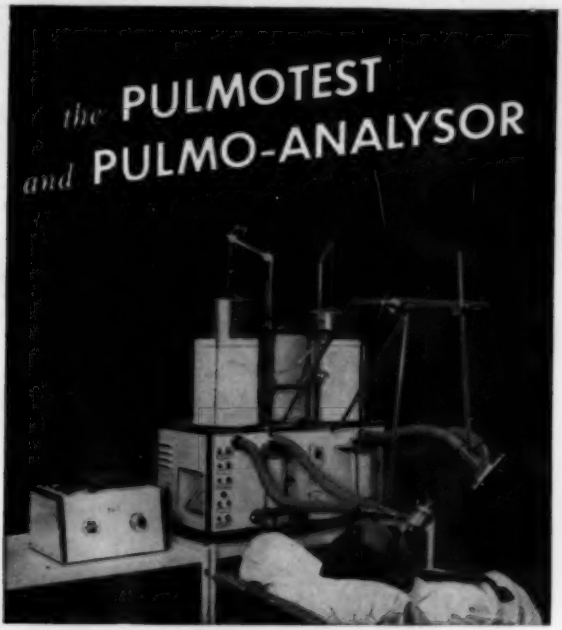
For further information and an application form, please write to the Executive Director, American College of Chest Physicians, 112 East Chestnut Street, Chicago 11, Illinois



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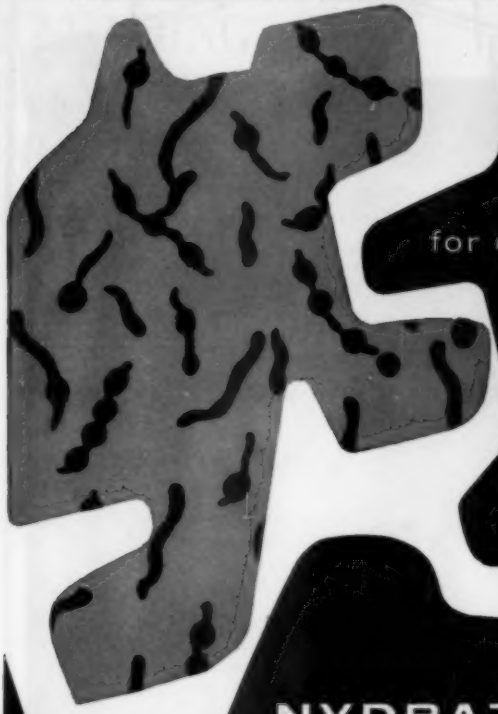

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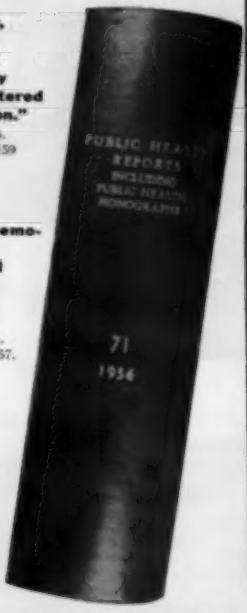

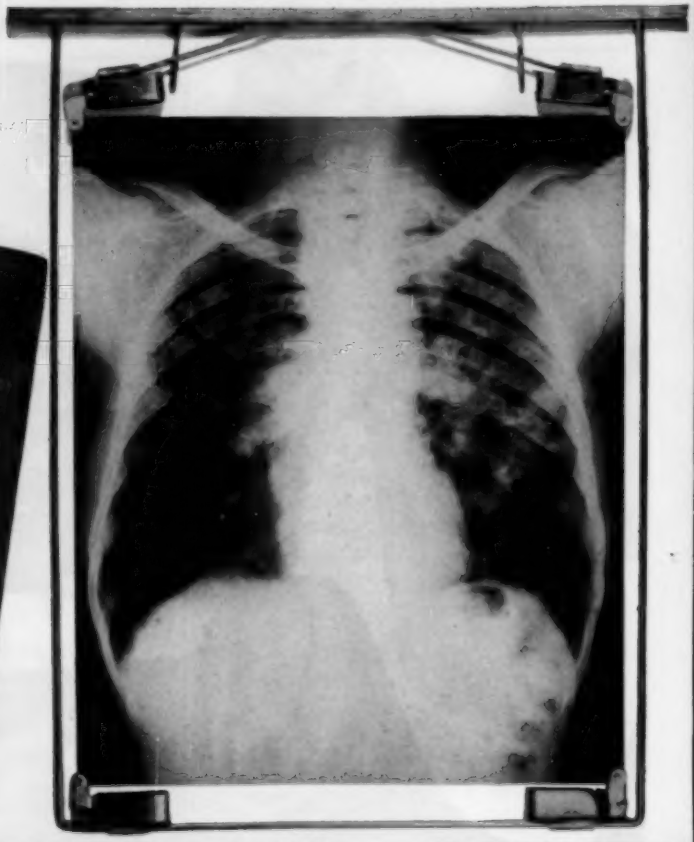

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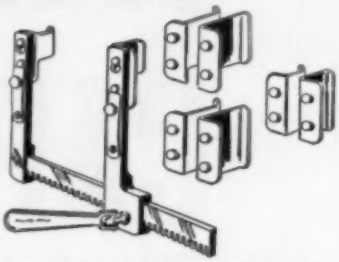
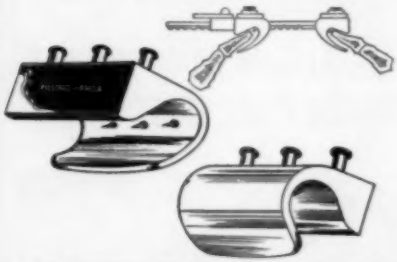


Th 224 Burford-Finochietto Infant-Child Rib Spreader and 4 pairs of interchangeable blades $98.00



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These sternal blades fit Pilling made Burford rib spreaders to provide the exposure necessary in transverse division of the sternum in a bilateral anterior thoracotomy, described in *J. Thoracic Surg.*, July 1957. Orders must specify model of rib spreader to be used in order to insure proper fit of blades.

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